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CONCERNING THE HYPERSENSITIVITY OF ADRENALECTOMIZED RATS TO VASCULAR STRESS[1]

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INTRODUCTION

ALTHOUGH IT IS well established that the adrenalectomized animal is hypersensitive to vascular stress, the compensatory mechanisms which fail in the absence of adreno-cortical hormones have not been directly demonstrated.

In normal intact animals, it is known that resistance to hemorrhage, as well as to other procedures which reduce the blood volume, is dependent in great part upon emergency reflex mechanisms which operate to maintain venous return and cardiac output. The efficiency of these compensatory adjustments may be appreciated from the finding that hemorrhage equal to 2.1 per cent of the body weight may not decrease venous return and cardiac output at all (Meek and Eyster, 1921), presumably because reserve blood from less active regions of the circulation is shifted to the active circulation.

The present work was undertaken to determine whether the hypersensitivity of adrenalectomized animals could be due to a primary failure of compensatory mechanisms for maintenance of venous return and cardiac output.

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METHODS

The rats used in these studies were obtained either from the colony of this laboratory or were Long-Evans rats: the animals received diets which have been shown to be satisfactory for both growth and reproduction over a period of years. In all experiments, care was taken to ensure that rats from the same strain, equivalent weight range and sex distribution were used in both the normal and adrenalectomized groups.

Following bilateral adrenalectomy, the animals received 1 per cent sodium chloride solution *ad lib.* and they were protected from extreme changes in the temperature of their surroundings. Five to seven days postoperatively, the rats were used for experimentation. As is known, salt-treated adrenalectomized rats show a surprisingly normal picture when maintained under protected conditions, but exhibit marked inadequacies in compensating against various types of stress (Evans, 1941).

Evaluation of venous return and cardiac output in normal and salt-treated adrenalectomized rats subjected to hemorrhage was obtained from the following considerations: when an animal is bled to death as rapidly as possible from the abdominal aorta, the amount of blood capable of being withdrawn is primarily dependent upon (a) the venous return to the heart and cardiac output, (b) the initial blood volume, and (c) the amount of interstitial fluid transferred across the capillary walls during the course of the bleeding.

By exsanguinating rats lying in the same relative position, the influence of gravity upon the bleeding volume values is constant. Since the arterial pressure at the portion of the aorta where blood is being withdrawn as rapidly as possible is almost immediately reduced to zero, and arterial blood is shunted to this point from all channels which offer resistance to blood flow, this means that the pressure of the arterial circulation, likewise, does not significantly influence the bleeding volume values.

The general plan of these experiments has been to measure the amount of blood capable of being withdrawn in a standardized acute exsanguination (henceforth called the bleeding volume) from normal and salt-treated adrenalectomized rats; and to evaluate the influence of factors *b* and *c* upon the values. This information being available, the effect of adrenal insufficiency upon venous return and cardiac output following hemorrhage is obtained by difference.

The procedures employed were the following:

Bleeding Volume

Etherized rats were placed on their backs, their abdomens opened and the intestinal contents pushed to one side. The floor of the abdomen was swabbed with absorbent cotton which served to remove peritoneal fluid and to expose the abdominal aorta. The needle of a previously weighed syringe was inserted into the aorta just above the ileac bifurcation, and blood was drawn into the syringe as long as it continued to flow. The needle with the largest gauge which could be inserted into the aorta was used. Following the removal of the syringe, the oozing of blood which occurs upon cutting the aorta was measured on tared absorbent cotton. The time interval for drawing blood with the syringe was approximately 60 seconds and 75–95 per cent of the bleeding volume is removed in this manner. The remaining small per-

centage is taken up by the tared cotton and approximately two minutes are required before bleeding from the severed aorta completely ceases. The following points observed in every case whether the animal was normal or adrenalectomized are important to note:

(a) It was observed that the heart continued to beat for at least one minute after flow from the severed aorta had ceased. This demonstrates that the bleeding volume values as measured in these experiments are not influenced by possible heart failure.

(b) The bleeding volume results obtained with the method described above are equivalent when similar values are obtained by cutting the abdominal aorta, and repeatedly sponging the blood upon tared cotton swabs. This means that a possible pressure effect of the arterial circulation upon the bleeding value by blood passing around the needle inserted in the aorta may be discounted.

(c) Upon cutting the beating heart of rats *after* bleeding volume has been determined, little or no oozing of blood occurs.

(d) The lungs, after exsanguination, are pale and ischemic.

Blood Volume

Average blood volumes were obtained by determining hematocrit and plasma volume in groups of normal and salt-treated adrenalectomized rats. The hematocrit values were determined on arterial blood drawn from the abdominal aorta of etherized rats following the procedure of Price and Longmire (1942). Plasma volume was measured with the T-1824 dye dilution method by adapting technical modifications suggested by Price and Longmire (1942) to the rat. The procedure used was as follows:

(1) A 1 cc. tuberculin syringe and snugly fitting 26 gauge needle were loaded with approximately 0.2 cc. per 100 gm. body weight of a 0.05 per cent solution of T-1824 dissolved in saline.

(2) The syringe, needle, and solution were then weighed to the closest milligram.

(3) The rat, which had previously weighed was etherized with minimal excitation, and one femoral vein was exposed.

(4) The needle of the loaded syringe was then thrust into the lumen of the vessel. The solution was injected and the dye was observed as it entered and mixed with the blood. After 30 seconds the syringe and needle were withdrawn and light pressure with a sponge prevented bleeding from the needle hole. The skin incision was then closed. (In any instance wherein dye leakage occurred during the course of the procedure, the animal was discarded.)

(5) The needle and syringe were wiped clean and dry, and were reweighed immediately. The difference in weight before and after injection represents the weight of dye solution given, and from this weight the amount of dye injected was calculated.

(6) After injection of dye, the etherized animal was allowed to awaken, and generally was walking about within 1–2 minutes. Since the time necessary to inject dye required only about 1 minute, this means that the animal was etherized for only about 3–4 minutes.

(7) After the dye had circulated for either 15 or 60 minutes, blood samples were drawn from the unanesthetized rat by heart puncture. The serum obtained was diluted with 1 per cent sodium chloride and dye concentration was determined by measuring absorption at 605 millimicra (correcting for

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hemoglobin by reading at 520 mμ) with a Beckman spectrophotometer. Plasma volume values uncorrected for dye disappearance from the blood stream during the interval of dye circulation were calculated from the dye dilution values; the mean percentage of dye lost per hour of circulation was calculated from the differences in dye dilution after 15 and 60 minutes of dye circulation. Finally actual plasma volume for normal and salt-treated adrenalectomized rats was obtained by extrapolation of the above data, assuming a linear rate of dye disappearance.

The following points of the above procedure should be emphasized:

(a) *Anesthesia.* McAllister (1938) has found in dogs that ether anesthesia decreases plasma volume an average of 11 per cent, generally within a few minutes after the induction of anesthesia, consequently the effect of anesthesia upon plasma volume estimation must be considered. In a search of the literature we have been unable to find good evidence for an anesthetic agent which does not alter plasma volume as estimated with modern methods. Nevertheless, anesthesia is necessary in the rat if dye is to be accurately injected. Without wishing to investigate the problem of anesthetics upon plasma volume in the rat in an attempt to discover some agent which would not alter plasma volume, we reasoned that in an animal etherized just long enough to permit dye injection, subsequently allowed to recover from anesthesia for 15 or 60 minutes, the plasma volume should be minimally affected by prior etherization. This idea was checked by demonstrating that rats etherized for 3–4 minutes, as described above, failed to show the hemoconcentration characteristic of ether anesthesia (Searles and Essex (1936) and others) 15 and 60 minutes after etherization as evaluated by hemoglobin determination on tail blood drawn before and after etherization.

(b) *Mixing Time.* Any determination of plasma volume by dye dilution methods is dependent upon extrapolating back to the time of dye injection and thus it is important to know when dye mixing is complete. Price and Longmire (1942) have discussed this problem in detail. The point, pertinent for this discussion, is that if one assumes dye mixing is complete in 5 minutes, then one estimates plasma volume to be lower (approximately 10–20 per cent) than if one decides that complete mixing requires 15–20 minutes. Following the opinion held by Gregerson and Stewart (1939) and by Gibson and Evans (1937) who believe that as long as 20 minutes are required for complete mixing, the assumption was made that complete mixing in our rats occurred in 15 minutes after dye injection.

Hemodilution during Course of Bleeding

The influence of interstitial fluid transfer through the capillaries can be considered negligible since it appears unlikely that significant hemodilution can occur during the 2–3 minute interval during which bleeding volume is obtained. Nevertheless, direct evidence on this point was obtained in the following manner: initial blood concentration comparisons were available from the hematocrit values in normal and adrenalectomized rats. Relative comparison of the extent of hemodilution during the course of the bleeding volume was made by determining the solids content of the complete blood samples obtained in the bleeding volume experiments. To determine percentage of solids, the blood was weighed initially and then dried to constant weight in the vacuum oven.

RESULTS

Bleeding Volume

Table I shows the bleeding volume of normal and salt-treated adrenalectomized rats. These data demonstrate that an average of 18 per cent less blood is obtained from salt-treated adrenalectomized rats using a standardized exsanguination technic. The lowering of bleeding volume following adrenalectomy is independent of the body

TABLE I. BLEEDING VOLUME OF NORMAL AND ADRENALECTOMIZED RATS (GM. BLOOD PER 100 GM. BODY WT.)

Normal rats			Adrenalectomized rats		
Exp. no.	Wt. and sex	Bleeding volume	Exp. no.	Wt. and sex	Bleeding volume
1	268 F	3.74	1	316 F	3.16
2	319 F	3.73	2	230 F	3.26
3	338 F	3.92	3	286 F	2.88
4	178 F	3.81	4	336 F	3.13
5	274 F	3.90	5	240 F	3.00
6	338 M	4.30	6	192 F	3.22
7	400 M	3.75	7	403 M	3.00
8	305 M	4.00	8	287 M	3.30
9	323 F	4.48	9	230 M	3.08
10	258 M	3.87	10	276 M	2.87
11	308 M	3.60	11	85 F	2.65
12	229 F	3.98	12	93 F	3.42
13	210 M	3.90	13	96 M	3.59
14	97 F	3.95	14	91 M	3.43
15	99 F	3.70	15	99 M	3.54
16	160 M	4.30	16	99 M	3.01
17	155 M	3.81	17	98 F	2.94
18	80 M	4.30	18	81 F	3.11
19	80 F	3.62	19	93 M	3.58
20	81 F	3.72	20	107 F	3.44
21	63 M	3.74	21	105 F	3.58
22	71 M	4.11	22	84 M	3.73
23	63 M	4.25			
24	99 F	3.94			
25	100 F	4.01			
26	200 M	3.85			
Mean		3.94			3.22
Standard Error		±0.14			±0.19

weight and sex. The differences are highly significant statistically. Although some variations obtain between individual experiments, the bleeding volume in no single experiment on adrenalectomized rats equals or exceeds the mean of the normal group. Moreover, the "t" value (Fisher, 1936) is 9.27 with a "P" value of 0.01.

To determine whether the decreased bleeding volume could be corrected by treatment with a cortical hormonal steroid, the effect of desoxycorticosterone upon the bleeding volume of salt-treated adrenalectomized rats was next determined. In these experiments, 5 mg. desoxycorticosterone acetate per 100 gm. body weight was ad-

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ministered 2 to 3 hours prior to experimentation.[4] The effect of treatment with desoxycorticosterone upon the bleeding volume of salt-treated adrenalectomized rats is shown on Table II. Table II also shows the results of similar desoxycorticosterone treatment on the bleeding values of normal rats, as well as comparative values of normal and adrenalectomized animals not treated with desoxycorticosterone acetate.

The results shown on Table II demonstrate that (a) the low bleeding volumes associated with adrenal insufficiency are raised to normal by desoxycorticosterone and (b) in the absence of adrenal insufficiency, *i.e.* in normal animals, desoxycorticosterone has no influence upon the bleeding volume. These findings, taken together, strongly

TABLE II. BLEEDING VOLUME OF NORMAL, ADRENALECTOMIZED, AND DESOXYCORTICOSTERONE-TREATED NORMAL AND ADRENALECTOMIZED RATS

Category	No. of rats	Mean bleeding volume	Standard error
		gm./100 gm. body wt.	
Normal	26	3.94	±0.14
Normal plus D.C.A.[1]	10	3.92	±0.10
Adrenalectomized	22	3.22	±0.19
Adrenalectomized plus D.C.A.[1]	10	3.93	±0.07

[1] 5 mg. desoxycorticosterone acetate per 100 gm. body wt. administered subcutaneously 2 to 3 hours prior to experimentation.

suggest that the lowered bleeding volume found in salt-treated adrenalectomized rats is due to an adrenal cortical hormone deficiency.

Blood Volume

The possibility that the decreased bleeding volume in salt-treated adrenalectomized rats is due to a reduced blood volume was checked by blood volume determination. Table III shows the plasma volume of normal and salt-treated adrenalectomized rats and Table IV the comparative hematocrit values. It is evident from these data that on the average, the blood plasma volume of salt-treated adrenalectomized rats is not significantly altered from the normal. The mean blood volume of both normal and salt-treated adrenalectomized rats as calculated from plasma volume and hematocrit is 8.9 cc. per 100 gm. body weight. The possibility that the lowered bleeding volume of salt-treated adrenalectomized rats is due to an initially lower blood volume is, therefore, not tenable.

[4] These conditions of hormone administration were selected because it has been found that desoxycorticosterone given under these circumstances restored to normal the markedly low resistance of salt-treated adrenalectomized rats to hind leg trauma. (Author's unpublished work.)

Hemodilution during Course of Bleeding

In Table IV, it was shown that the hematocrit of salt-treated adrenalectomized rats is normal. If normal rats have higher bleeding volume values than adrenalectomized rats because of increased hemodilution with interstitial fluid, then the blood sample obtained in the

TABLE III. THE PLASMA VOLUME OF NORMAL AND ADRENALECTOMIZED RATS

Dye circulation time	Category	No. of rats	Average wt.	Plasma volume (uncorrected) including standard error	Plasma volume[1] (corrected)
min.			*gm.*	*cc./100 gm. body wt.*	*cc./100 gm. body wt.*
15	Normal	10	280	5.09 ± 0.07	5.01
	Adrenalectomized	10	300	5.08 ± 0.06	4.98
60	Normal	8	278	5.35 ± 0.08	5.00
	Sham operated	8	296	5.33 ± 0.08	4.98
	Adrenalectomized	10	304	5.40 ± 0.08	4.97

[1] Corrected for dye loss. Calculation reveals 6.5% and 7.9% dye loss per hour of circulation for normal and adrenalectomized rats respectively.

TABLE IV. THE HEMATOCRIT OF NORMAL AND ADRENALECTOMIZED RATS

Category	No. of rats	Average wt.	Average hematocrit	Standard error
		gm.		
Normal	10	322	0.44	±0.01
Adrenalectomized	12	302	0.44	±0.01

TABLE V. THE PERCENTAGE SOLIDS IN THE BLOOD OF NORMAL AND ADRENALECTOMIZED RATS

Category	Average wt.	Number of rats	Average solids	Standard error
	gm.		*per cent*	
Normal	294	10	18.7	±0.2
Adrenalectomized	280	10	18.6	±0.2

measurement of bleeding volume should contain a significantly lower percentage of solids. (Interstitial fluid is low in solids content as compared to whole blood, approximately 1:18.) Table V shows the percentage of solids in the complete blood samples obtained in the bleeding volume experiments on normal and salt-treated adrenalectomized rats. It will be seen that there are no significant differences between the groups. This finding taken, together with the initial hemat-

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ocrit data, demonstrates that the lowered bleeding volume observed in adrenal cortical insufficiency is not explicable in terms of decreased transfer of interstitial fluid to the blood stream.

DISCUSSION

It has been demonstrated in this study that the amount of blood obtainable from rats by a standardized acute exsanguination is significantly decreased following adrenalectomy. This difficulty in blood mobilization has been shown not to be due to an initially lower blood volume or to a decreased shift of interstitial fluid into the blood stream during the course of bleeding. Since the method employed does not appear to be influenced by the pressure of the arterial circulation, possible differences in the initial arterial blood pressure between normal and salt-treated adrenalectomized rats likewise cannot explain the lowered bleeding volume. It therefore appears that the decreased bleeding volume reflects a reduced venous return and cardiac output in exsanguinated salt-treated adrenalectomized rats.

The findings in the adrenal-deficient rat following determination of bleeding volume (after flow of blood from the opened aorta has ceased) that (a) the heart continues to beat for some time, (b) the lungs are not congested but are pale and ischemic, and (c) blood does not flow from the sectioned heart, taken together provide strong evidence that the difficulty in blood mobilization is primarily due to decreased venous return and not to primary failure of cardiac output.

Since the above conclusions are wholly dependent upon the finding that salt-treated adrenalectomized rats have normal plasma and blood volumes, it is necessary that the accuracy of our plasma volume method should be completely satisfactory. The values reported in this study for normal plasma volume appear to be high as compared to other estimates of plasma volume using T-1824 in the rat. Thus Metcoff and Favour (1944) find a plasma volume of 2.8 cc. per 100 cm.^{2} of body surface, while our values in terms of 100 cm.^{2} body surface are 3.8 cc. These differences, however, aside from the differences in strain and laboratory condition of the rats used, may perhaps be explained on the following basis: (a) Metcoff and Favour determined plasma volume on etherized rats, while in this study the effect of prior etherization may be considered minimal. If the plasma volume of the rat is decreased by ether to the same extent as occurs in dogs (McAllister (1938)), then the plasma volume values of Metcoff and Favour (1944) would be 6.3 to 17.2 per cent, with an average of 11 per cent, lower than those on unanesthetized rats. (b) Metcoff and Favour (1944) assume that complete mixing occurs in approximately 4 minutes with insignificant dye loss and consequently do not extrapolate. In this study, following the interpretation of Gregerson and Stewart (1939) and of Price and Longmire (1942), it has been assumed that complete mixing requires 15 minutes. The extrapolated

values, as has been mentioned previously, give rise to higher estimates of plasma volume in the order of 10 to 20 per cent. In this work it has been assumed that dye disappearance follows a linear curve. However, recent studies (King *et al.* (1943) and Gregerson and Rawson (1943)) indicate that dye disappearance may be best described on the basis of an exponential type of curve. According to King *et al.* (1943), plasma volume values calculated by their square root extrapolation method for any period over 10 minutes of dye circulation average 10 per cent less than those calculated by linear extrapolation.

In considering the above, it would appear that the differences between the values obtained in this study and those of Metcoff and Favour (1944) are more apparent than real. As regards the question as to which values are the "true" estimates, it becomes a question of choosing between various assumptions and only secondarily concerns us here. The important point is that salt-treated adrenalectomized rats have the same dye dilution at two different points as do normals. Regardless, then, of methods of extrapolation, it would follow that salt-treated adrenalectomized rats have normal plasma volume, whatever that value may be as the result of introducing assumptions as to the curve of dye disappearance, complete mixing time, etc. It may be mentioned that the finding that salt-treated adrenalectomized rats have normal blood volume is not entirely new. Evans (1941) working with normal and salt-treated adrenalectomized rats estimated blood volume to be 7.0 and 6.9 per cent of the body weight respectively, using brilliant vital red. These estimates, however, were obtained on anesthetized, kidney-ligated, eviscerated (but not hepatectomized) groups of rats. The details of his data were not given.

The decreased mobilization of blood in bled salt-treated adrenalectomized rats would appear to be a primary effect of adrenal insufficiency since treatment of adrenalectomized rats with salt solution prevents a terations of blood electrolyte levels, hemoconcentration, loss of weilght, anorexia (Evans, 1941). That the difficulty in blood mobilization is due to an adreno-cortical hormone deficiency seems evident, since desoxycorticosterone acetate raises the low bleeding volume of adrenal deficient rats to normal levels, but has no effect on bleeding volume in the absence of adrenal insufficiency.

The results obtained in this study may serve to explain the causation of the hypersensitivity of adrenalectomized animals to vascular stress. Since the factors which determine bleeding volume (with the exception of venous return and cardiac output) are equivalent in normal and salt-treated adrenalectomized rats, the magnitude of reduction in bleeding volume following adrenalectomy may be taken as a measure of the reduction in venous return and cardiac output. On this basis, venous return and cardiac output following acute exsanguination are reduced an average of 18 per cent. This would mean that a fraction of the total circulating blood volume (equivalent to 18

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per cent of the blood volume or 1.6 per cent of the body weight in blood) available for active circulation in normal rats cannot be thus mobilized in the absence of adrenal cortical hormones. Comparative evaluation of resistance to hemorrhage indicates that rapid bleeding equivalent to 3.5 per cent of body weight kills approximately 50 per cent of etherized normal rats, while rapid removal of 2.0 per cent of the body weight of blood kills approximately 50 per cent of etherized salt-treated adrenalectomized rats (author's unpublished observations). This critical bleeding of 2.0 per cent of body weight for adrenal insufficiency is in agreement with the data of Evans (1941) who found that this hemorrhage level kills a significant percentage of salt-treated adrenalectomized rats, although the hemorrhage necessary to kill 50 per cent of his group was not reported. If one accepts the calculated value for the amount of blood not mobilized for compensatory maintenance of venous return and cardiac output following adrenalectomy, the lowered resistance of adrenalectomized rats maintained with salt solution could be explained solely on the basis of this factor.

Whether or not these deductions are quantitatively applicable to the decreased resistance following slow, repeated bleedings or to other vascular stresses in rats and other species is unknown. However, it does appear likely that the failure of a venopressor mechanism suggested from these studies participates qualitatively in the development of hypersensitivity following adrenalectomy in these situations.

SUMMARY

The amount of blood capable of being withdrawn in a standardized acute exsanguination (bleeding volume) is reduced significantly in salt-treated adrenalectomized rats. This difficulty in blood mobilization appears to be the effect of a deficiency in a cortico-adrenal hormone since desoxycorticosterone acetate raises the bleeding volume of adrenalectomized rats to normal levels, but is without influence on the bleeding volume of normal animals. In interpreting the significance of these findings the conclusion is drawn that lowered bleeding volume reflects a decreased venous return and cardiac output in bled adrenalectomized rats since the lowering of bleeding volume cannot be ascribed to decreased blood volume, decreased interstitial fluid transfer, or to possible effects of the pressure of the arterial circulation. The absence of heart failure or lung congestion, and the failure to observe bleeding from the cut heart of adrenalectomized rats after bleeding volume has been determined is strong evidence that the decreased bleeding volume is primarily due to reduction of venous return. This would mean that the adrenal cortical hormones are involved in the operation of compensatory venopressor mechanisms. The significance of a primary failure of a venopressor mechanism as an explanation for the hypersensitivity of adrenalectomized animals to vascular stress has been discussed.

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TIME RELATIONSHIPS OF DECIDUOMATA FORMATION IN PROLACTIN-TREATED AND NORMAL PSEUDOPREGNANT RATS

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THE DECIDUOMAL reaction, first described by L. Loeb (1907), has served as an important criterion for luteal activity since Weichert's demonstration (1928) that estrogen and progestin, acting in sequence, are necessary for its production. Since in normal rats the reaction may be evoked only during pseudopregnancy and not during the cycle (Long and Evans, 1922), and since injections of pituitary extracts permit the production of the deciduomal reaction at other times the theory has been advanced that a luteotrophic substance occurs in the pituitary (Astwood, 1941; Evans, Simpson, Lyons and Turpeinem, 1941). The present experiments were designed to test whether injections of the pituitary luteotrophic material such as that associated with lactogenic hormone preparations would hasten the time that deciduomal reactions could be evoked.

MATERIALS AND METHODS

Daily vaginal smears were taken on 56 young, mature, nulliparous rats until it was established that their estrous cycles were regular. The animals were then divided into three groups on the basis of pre-operative treatment or hormone injection.

A general description of the operative procedure, post-operative interval and autopsy routine can be given since they were identical for each animal. The animal was anesthetized with ether and the uterus traumatized by the introduction of two silk threads into the lumen of the left cornu. A post-operative period of 120 hours was allowed before necropsy. After inspection for the presence of deciduomata, the uterus was fixed in Bouin's solution preparatory to histological study. All uteri which did not show deciduomata on inspection were serially sectioned at 10 micra, stained with Delafield's hematoxylin and examined microscopically.

Group I: Thirty-one animals were given daily subcutaneous injections of 10 to 60 I.U. prolactin (L.H.395) in 0.1 cc. distilled water from the day of vaginal estrus through the fourth day after uterine traumatization. The uteri were traumatized 24, 48 or 72 hours after the initial injection of hormone.

Group II: The period of estrus for seven rats was determined by the copulatory response method (Blandau, Boling and Young, 1941) and found to occur when the vaginal smear showed a predominance of nucleated epithelial cells. When the animals gave a positive copulatory response, daily injections of 20 I.U. prolactin in 0.1 cc. distilled water were begun and continued through the fourth post-operative day. The uteri were traumatized on the day of vaginal estrus, 24 hours following the initial injection of hormone.

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Group III: The period of estrus for eighteen rats was established by the copulatory response method. At the time of estrus (vaginal pro-estrus) the animals were caged overnight with vasectomized males. Uterine traumatization was performed 24, 48, 72, 90 or 120 hours after the animals were caged together.

RESULTS

The results are summarized in tables I and II.

Group I: This group includes all animals to which an initial injection of hormone was administered during vaginal estrus. In five of twelve animals microscopic deciduomata occurred as the result of endometrial injury on the first day of diestrus. Macroscopic deciduomata of moderate size were present in the uteri of nine of ten animals operated on the second day, while traumatization on the third diestrous day evoked large deciduomal growths at all loci of injury.

No direct relationship between hormone concentration and deciduomata formation could be established for this group of animals.

Group II: With the exception of one small decidual tumor no deciduomata could be detected on macroscopic examination of the uteri of the animals given an initial injection of prolactin during

TABLE I. EFFECT OF PROLACTIN ON DECIDUOMATA IN RESPONSE TO SUTURE TRAUMA.

Group	No. of rats	Prolactin		Uterine traumatization after injection	Deciduomata	
		Daily dose	Time of initial injection		Positive	Negative
I	1	10 I.U.	During Vaginal Estrus	24 hrs.	0	1
	2	20 I.U.		24 hrs.	1	1
	7	40 I.U.		24 hrs.	4	3
	2	60 I.U.		24 hrs.	0	2
	1	10 I.U.	DuringVaginal Estrus	48 hrs.	1	0
	2	20 I.U.		48 hrs.	2	0
	5	40 I.U.		48 hrs.	4	1
	2	60 I.U.		48 hrs.	2	0
	1	10 I.U.	During Vaginal Estrus	72 hrs.	1	0
	2	20 I.U.		72 hrs.	2	0
	5	40 I.U.		72 hrs.	5	0
II	7	20 I.U.	During Vaginal Proestrus	24 hrs.	6	1

TABLE II. EFFECT OF COPULATION WITH VASECTOMIZED MALE ON DECIDUOMATA FORMATION IN RESPONSE TO SUTURE TRAUMA

Group	No. of. rats	Day mated	Time of traumatization after copulation	Deciduomata	
				Positive	Negative
III	7	During Vaginal Proestrus (Physiological Estrus)	24 hours	4	3
	6		48 hours	6	0
	2		72 hours	2	0
	2		90 hours	2	0
	1		120 hours	1	0

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physiological estrus and operated on the day of vaginal estrus. The traumatized cornua appeared slightly larger than the controls, but were uniform in diameter. When examined microscopically, however, small but typical deciduomata were found in six of seven animals.

Group III: In the normal pseudopregnant animals deciduomata were present in four of seven uteri traumatized within 24 hours after copulation. In two of the uteri the presence of deciduomata could be determined by gross inspection. When the interval between caging and operation was 48 hours, a 100% deciduomal response was obtained. Deciduomata in four of the six uteri could be ascertained by macroscopic examination. In all uteri traumatized at a later date deciduomata were present, which were, however, considerably smaller than those observed for the prolactin-treated rats operated on the third day of diestrus.

DISCUSSION

Several lines of evidence (Lahr and Riddle, 1936; E. Cutuly, 1941; Evans, Simpson, Lyons and Turpeinem, 1941; Pfeiffer, 1941) indicate that the lactogenic principle of the hypophysis is the luteotrophin responsible for the maintenance of functional corpora lutea in the rat. A neuro-humoral mechanism may be involved in evoking its elaboration (Greep and Hisaw, 1938; Astwood, 1941; Zacharias, 1941).

The results obtained in the present investigation support the existing evidence. When the initial injection of prolactin is given during vaginal estrus and the uteri subsequently traumatized, the percentage of positive reactions, as well as the size of the tumors, is directly correlated with the length of the pre-trauma period (the interval between initial hormone injection and traumatization). When the hormone is first administered during physiological estrus (vaginal pro-estrus), microscopic deciduomata occur as the result of endometrial trauma on the day of vaginal estrus. Since the percentage of positive responses in the latter instance is significantly higher (85.7%:41.6%), it is concluded that the ovary is optimally sensitive to the lactogenic preparation at the time of ovulation.

Traumatization of the uteri of normal pseudopregnant rats 24 or 48 hours following copulation evokes the deciduomata reaction, the percentage of positive responses being 57.1% and 100% respectively. This phase of the investigation is subject to criticism inasmuch as the time selected for endometrial trauma was based on the assumption that mating occurred immediately after the female and male were caged together. Although it is possible that some of the operations were performed as early as 14 hours following copulation, the three negative responses cannot be attributed to this fact alone until more controlled experiments are performed. Individual variation in the quantitative elaboration of the luteotrophin or progestin, differences in the threshold of endometrial response, and the possibility of potentiation by estrogen must also be considered.

Since microscopic deciduomata will form as the result of endometrial injury on the day of vaginal estrus in prolactin-treated and normal pseudopregnant rats, it is concluded that the lactogenic principle of the anterior pituitary is elaborated in response to a stimulus set up by cervical stimulation. The correlation of tumor size and frequency of occurrence with the length of the pre-trauma interval further indicates that uterine sensitization is a quantitative phenomenon occurring earlier in pseudopregnancy than has been previously reported.

SUMMARY

Daily administration of 10 to 60 I.U. prolactin (beginning on the day of vaginal estrus) to normal cyclic rats brings about a positive deciduomata reaction to endometrial trauma on the first, second and third days of diestrus. The frequency of occurrence and size of the deciduomata are correlated directly with the length of the interval between initial hormone injection and endometrial injury.

When the initial injection of hormone is administered during physiological estrus (vaginal pro-estrus), microscopic deciduomata occur in response to endometrial trauma on the day of vaginal estrus. Traumatization of the endometria of normal pseudopregnant rats within 24 hours after copulation evokes a similar response in a lower percentage of cases (85.7%:57.1%).

The experimental evidence indicates that: (1) the ovary is most sensitive to injected lactogenic hormone at the time of ovulation; (2) uterine sensitization, as measured by the deciduomata reaction, is a quantitative phenomenon and appears at a time earlier than that reported by other investigators; and (3) the lactogenic principle of the anterior pituitary may be the hormone responsible for the initiation of function of corpora lutea following cervical stimulation in the rat.

ACKNOWLEDGMENTS

The author is indebted to Dr. C. K. Weichert, who suggested this problem for investigation, for much valuable criticism. Thanks are also due Dr. C. W. Barlow of Winthrop Chemical Company for the generous supplies of prolactin used in the investigation.

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DEMONSTRATION OF A CYCLIC CHANGE IN THE HISTOCHEMISTRY OF THE KIDNEY SYNCHRONIZED WITH THE ESTROUS CYCLE IN THE RAT[1]

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ALDEHYDES in the tissues were demonstrated by their reaction with Fuchsin Sulfurous Acid (FSA) by Feulgen and Voit (1924). They were found to consist mainly of stearal and palmital linked in a cyclic acetal bond to glycerol-phosphoryl-cholamine with the following structure (Feulgen and Bersin, 1939): $RHC\underline{-OCH_2-CHO-CH_2-OPO(OH)-OCH_2-CH_2-NH_2}$. They are present in various tissues in most animals. Oster and Mulinos (1944) showed that tissue aldehydes exhibit a characteristic distribution pattern in kidneys of rats, guinea pigs, rabbits, cats and dogs, when stained with FSA.

In the cortex all glomeruli stain lightly, and only isolated tubules stain deeply, those remaining being almost unstained. The deepest and most uniform staining is seen in the intercortico-medullary zone (ICM zone). The Henle's loop region of the medulla stains less deeply, and groups of unstained collecting tubules give a spoke-like appearance to the loop bundles. The pelvic portion of the medulla is almost unstained. Use of the FSA stain, therefore, provides an excellent method for differentiating between these various functional units of the kidney (Fig. I).

This distribution pattern was uniformly found in the 42 male rats, both unoperated and operated, which were used in the present study. In 68 female animals this pattern was principally the same. However, the distribution of the tissue aldehydes in the intercortico-medullary zone was altered. This change was made the subject of investigation.

METHODS

The experimental procedures used were essentially the same as those described by Oster and Mulinos (1944). Findings in both kidneys were consistently the same at a given time.

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[1] A preliminary report of this work was delivered before the Section of Biological Chemistry of the American Chemical Society at its Semi annual Meeting in New York City, September 15, 1944.

EFFECT OF ADRENALECTOMY AND SPLENECTOMY

The effect of adrenalectomy on the aldehyde distribution pattern was studied in 17 male and 21 female animals, and no change was found in males for as long as 30 days after operation. Female rats, on the other hand, showed definite staining pattern changes in the intercortico-medullary zone. Five days after operation a definite destaining was noticeable in the portion of the zone which is closest to Henle's loops, and 15 days post-operatively almost the entire zone was de-

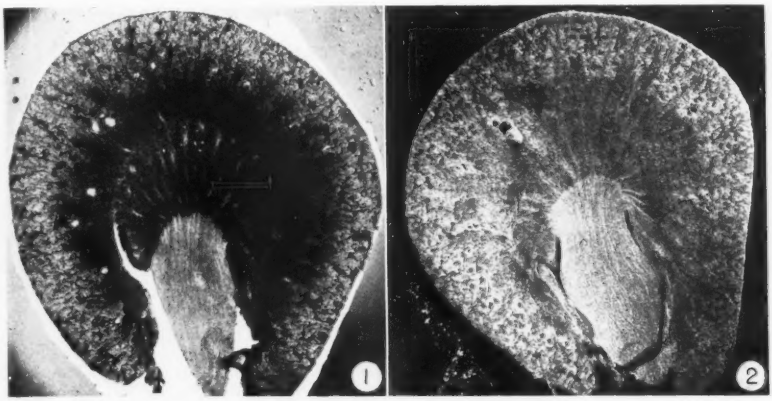

FIG. 1. Kidney of female rat in estrus. Note four zones of varying staining intensity: a. cortical zone with stained isolated tubules and glomeruli. b. intercortico-medullary zone, intensely stained. c. Henle's loop zone, interrupted by unstained collecting tubules. d. pelvic zone, almost unstained. Fuchsin Sulfurous Acid stain. ×8.

FIG. 2. Kidney of pregnant rat. Compare the greatly diminished staining intensity in the intercortico-medullary zone with that shown in Fig. 1. Fuchsin Sulfurous Acid stain. ×8.

stained. Daily intramuscular injections of 10 mg. desoxycorticosterone acetate in oil[2] for 12 days and daily intramuscular injections of 25 dog units of adrenal cortical extract[3] for 10 days into adrenalectomized female rats did not prevent the destaining of the intercortico-medullary zone from taking place.

In order to ascertain whether the observed effect was specific for adrenalectomy or was merely a result of the operative procedure, splenectomies were performed on 6 male and 5 female animals. In the male kidneys no histochemical changes occurred, but female kidneys displayed a progressive destaining in the intercortico-medullary zone, similar to that seen in the adrenalectomized animals. Thus, a specific influence of adrenalectomy on the histochemical distribution of the tissue aldehydes was ruled out.

[2] We wish to thank Dr. Erwin Schwenk, Schering Corporation, for supplying us with desoxycorticosterone acetate.

[3] Eschatin, Parke, Davis.

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EFFECT OF PREGNANCY

Since it is known that operative procedures cause a state of pseudopregnancy in female rats (Griffith and Farris, 1942), it was postulated that this occurrence might be the cause of the observed changes. Examination of the kidneys of 3 pregnant animals showed an almost complete disappearance of the aldehydes from the intercortico-medullary zone (Fig. II). One of these females had undergone a unilateral nephrectomy. Thirty-four days after bearing her litter, an examination of the vaginal smear showed her to be in the estrous phase of the cycle. Her remaining kidney exhibited, on examination, a deeply stained intercortico-medullary zone, a complete reappearance of the tissue aldehydes in this region.

EFFECT OF ESTROUS CYCLE

Starting from this observation, the kidneys of 37 normal female animals were examined, after determining the phase of the estrous

TABLE I.

PHASES OF CYCLE

Findings in ICM zone	Proestrus-estrus estrus estrus-metestrus metestrus	Metesterus-diestrus diestrus diestrus-proestrus proestrus
+ +	5	—
(+) +	4	—
(−) +	3	—
− +	6	7
− (+)	—	6
− (−)	—	4
− −	—	2
	18	19

ALDEHYDE DISTRIBUTION IN THE INTERCORTICO-MEDULLARY (ICM) ZONES OF 37 FEMALE RATS IN THE ESTROUS AND DIESTROUS PHASES OF THE CYCLE.

cycle of the individual animal at the time it was either nephrectomized or sacrificed. On collecting all findings, it was observed that normal female rat kidneys differed considerably one from the other in staining intensity of the intercortico-medullary zone.

It was thus necessary to construct an arbitrary grade scale for the staining quality of this area. The intercortico-medullary zone was divided into two halves. When both halves were deeply stained, it was designated as a ++ staining reaction. A − − pattern denoted a destaining of both halves. Between these two extremes various degrees of destaining were found. Since most of the changes occurred in the medullary half of the zone, 7 of the 16 possible combinations were used, ++, (+)+, (−)+, −+, −(+), −(−), and − −.

The 37 animals were divided into two groups, one consisting of 18 rats in the follicular phase of the estrous cycle and the other consisting

of 19 rats in the corpus luteum phase of the cycle. All animals in the estrous phase, ranging from proestrus excluded to metestrus included, had $-+$ to $++$ staining patterns. On the other hand, all animals in the diestrous phase, ranging from metestrus excluded to proestrus included, showed staining patterns ranging from $-+$ to $--$ (Table I).

In a normal population of 37 female rats one cannot expect to find offhand 18 animals in the estrous and 19 in the diestrous phase of the cycle. To obtain this distribution the animals were operated upon or sacrificed, after taking several vaginal smears until the desired phase of the cycle was reached. The smear was not always found to be in agreement with the state of the ovaries, which were carefully examined in each animal. Therefore, the state of the ovaries was made the determining factor in designating the respective estrous phases.

The material presented in Table I together with the above results with the pregnant animals suggested a cyclic existence of the aldehydes in the intercortico-medullary zone. In the estrous phase the aldehydes tend to increase, and in the diestrous phase they tend to decrease.

EFFECT OF OVARIECTOMY

There could be other glandular influences, aside from the ovaries, which might be responsible for this change. Therefore, the kidneys of spayed animals were examined. After establishing the fact that a carefully performed unilateral nephrectomy had no lasting influence on the estrous cycle or on the staining behavior of the intercortico-medullary zone in the remaining kidney, 6 animals in various phases of the cycle were simultaneously nephrectomized and ovariectomized. An examination of the staining pattern of the excised kidney was made on the day of operation and the staining of the remaining kidney noted 6 or 12 days postoperatively. Invariably, the remaining kidney was in almost the same state as the first operated one. The only difference noted was that as far as the intercortico-medullary zone was concerned, it completed the phase of the cycle which would have developed, if the ovaries were present to exert their influence. This effect was ascribed to the influence of hormones already present in the tissues at the time the ovariectomy was performed.

Three animals were simultaneously ovariectomized and adrenalectomized. They failed to show a destaining reaction 2 weeks after operation.

These results make it doubtful that any gland other than the ovaries influences the observed phenomenon in the intercortico-medullary zone. For additional evidence the fact is cited that kidney examination of 5 young females with immature ovaries and closed vaginas showed a $++$ staining reaction. In these animals the corpus luteum did not yet have a chance to exert its influence on the kidney.

Disregarding certain overlappings which one would necessarily

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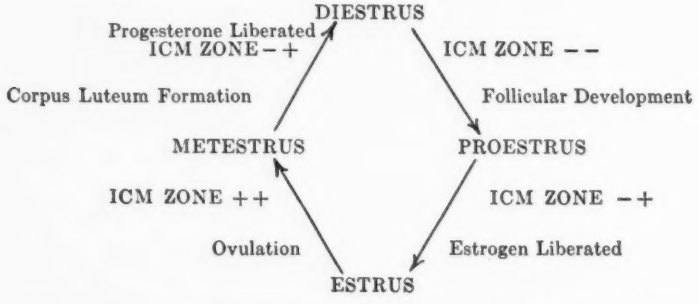
expect in such a short cycle of 5 days duration in the rat, the following scheme is suggested, showing the ideal conditions in which the state of the ovaries and aldehyde content of the intercortico-medullary zone run parallel (Table II).

EFFECTS OF INJECTION WITH ESTROGENS AND PROGESTERONE

Preliminary experiments have been carried out to determine whether the described influence is one of the known estrogen or progesterone hormones. Normal females in the diestrous phase were in-

TABLE II.



RELATIONSHIP BETWEEN THE ESTROUS CYCLE AND THE INTERCORTICO-MEDULLARY (ICM) ZONE OF THE RAT KIDNEY

jected with estradiol. A + + reaction was not obtained in the kidney after 6 intramuscular injections of 0.05 mg. each over a period of 3 days. This does not necessarily imply that estradiol is not the hormone which is responsible for the observed changes. It may mean that in the ovary, thus stimulated, is present the stronger influence of another hormone which prevents the changes from taking place.

Therefore, either progesterone (1.5 mg. daily) or estradiol (0.05 mg. daily) were given intramuscularly to orchidectomized or orchidectomized-adrenalectomized animals. Despite a 4 day injection period with these hormones, no changes from the + + state of the intercortico-medullary zone were noticed.

The evidence obtained so far leads one to believe that possibly hormones produced in the corpus luteum other than progesterone might be responsible for the occurrence of this new phenomenon. The possible existence of such corpus luteum hormones has been cited in the literature (Fevold *et al.*, 1932, Freed and Soskin, 1938).

DISCUSSION

One can assume a chemical rather than a structural change of the kidney cells, since the latter cannot be demonstrated with ordinary staining methods. The chemical reactions which may cause the in-

ability of the fuchsin sulfurous acid reagent to combine with the tissue aldehydes are manifold. For instance, the aldehydes might change their acetal linkage and form another combination which resists scission by mercuric chloride. Or they may be oxidized or reduced to the respective acid or alcohol.

The physiological significance of the disappearance of aldehydes from the intercortico-medullary zone during the diestrous phase of the cycle is not known as yet. The disappearance of one chemical group in the cells of such a carefully balanced structural unit as the kidney should have some physiological effect. It might influence the urinary secretion and reabsorption which is believed to take place in that particular portion of the kidney. Compensatory processes could, however, mask any such actual manifestations in the urine. The possibility arises that such changes in aldehyde composition might also take place in other organs of the body. There is much clinical evidence in humans linking a disturbed water and electrolyte balance with ovarial activity and kidney function, especially during pregnancy and premenstrual tension.

SUMMARY

Tissue aldehydes exhibit a characteristic distribution pattern in the rat kidney.

A change of the tissue aldehydes in the intercortico-medullary zone of the rat kidney concomitant with the estrous cycle has been described. During estrus the aldehydes tend to increase, and during diestrus they decrease. A scheme is presented showing the ideal conditions of the phenomenon. Ovarial hormones alone influence the state of the aldehydes in the intercortico-medullary zone. Progesterone might not be the hormone responsible for the disappearance of the aldehydes in the intercortico-medullary zone.

The chemical and physiological significance of the phenomenon is discussed.

We wish to thank Mr. A. Macovsky for the photomicrography.

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INFLUENCE OF THIOUREA ON PLASMA PROTEINS AND ORGAN WEIGHTS IN THE RAT[1,2]

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HYPOPHYSECTOMY in the rat is followed by a rise in serum globulin concentration and a decrease in serum albumin whereas thyroidectomy is followed by a rise in serum globulin but the albumin concentration remains unchanged. (Levin and Leathem, 1942; Levin 1943). Administration of thyroxine to the hypophysectomized rat prevents the rise in serum globulin without preventing the decrease in serum albumin thus indicating a relationship between thyroid function and serum globulin concentration (Levin and Leathem, 1942). Since it is well established that thiourea will induce hypertrophy of the thyroid and is thought to prevent the formation of thyroid hormone (MacKenzie and MacKenzie, 1943; Astwood, Sullivan, Bissell and Tyslowitz, 1943; Astwood, 1943; Keston, Goldsmith, Gordon and Charipper, 1944; Franklin, Lerner and Chaikoff, 1944), it appeared of interest to determine whether feeding thiourea would result in blood protein changes similar to those induced by thyroidectomy. This method would reflect any changes in the albumin-globulin ratio without the side effects, possibly resulting from surgical procedures. The influence of thiouracil on total serum protein and nitrogen balance of patients with Graves' disease has been investigated but no data on albumin-globulin concentrations were reported (Williams and co-workers, 1944 (a); Sloan and Shorr, 1944).

Hypertrophy of the thyroid is invariably observed following the administration of thiourea or its derivatives despite impaired growth in young rats (Astwood, Sullivan, Bissell and Tyslowitz, 1943; Hughes, 1944; Williams, Weinglass, Bissell and Peters, 1944). Other organs such as the gonads, spleen, kidney and liver are not influenced by the drugs but whether or not the adrenals and pituitary are altered is as yet controversial. Kennedy and Purves (1941) found that Brassica seed caused an hypertrophy of the adrenal whereas Richter and Clisby (1942) and Williams and co-workers (1944 b) found the adrenal unchanged following phenyl thiourea or thiouracil adminis-

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[2] Grateful acknowledgement is made to Dr. J. B. Allison for criticism and supervision of chemical aspects of this problem and to E. Bartner for valuable technical assistance.

tration. Several investigators have described pituitary changes resulting from the administration of goiterogenic drugs causing an increase in basophiles and a decrease in eosinophiles (Griesbach, 1941; Kennedy, 1942; MacKenzie and MacKenzie, 1943) whereas Williams and co-workers (1944 b) observed no changes in pituitary cytology following thiouracil. It appeared of value to determine the effect of thiourea on the organ weights and also to test the gonadotrophic hormone potency of the pituitary for evidence of an altered physiologic state.

An influence on total white cell counts of circulating blood has been observed with thiouracil in some cases (Williams and co-workers, 1944 b) and additional information on this point in reference to thiourea is presented in this report.

MATERIALS AND METHODS

Male rats of the Long-Evans strain, ranging from 124 to 147 days in age, were used. The animals were kept in pairs in metabolism cages for measurement of daily food intake. The stock diet of purina calf meal plus 10 per cent meat scrap, the latter containing 55 per cent protein, was available at all times. This diet was supplemented by a mixture of $\frac{2}{3}$ mazola and $\frac{1}{3}$ cod liver oil on bread twice weekly and by fresh carrots once each week.

Thiourea was fed as one per cent of the stock diet and after an experimental period of 20–22 days the rats were lightly anesthetized with ether and bled from the heart. Heparin was used as anticoagulant in a concentrated solution and 0.1 cc. was held in the syringe while 8 to 12 cc. of blood were drawn from the heart without opening the thoracic cage. A small portion of the blood was used for hematocrit determination (Wintrobe, 1929). After centrifugation the plasma was used for determination of proteins by the Pregl micro-Kjeldahl method. The albumin and globulins were separated by the method of Howe (1921) as modified by Robinson, Price and Hogden (1937). Non-protein nitrogen was determined on filtrates following precipitation with 20 per cent trichloracetic acid. The nitrogen values were corrected for NPN and converted to protein by use of the factor 6.25. Globulin was calculated as the difference between total protein and albumin.

Heparin was not used when blood was drawn from the heart for total red and white cell counts.

Each rat was autopsied and fresh weight of the carefully dissected pituitary, adrenals, thyroids, testes, seminal vesicles (including the coagulating gland and any contained fluid), ventral prostate, spleen, liver and kidney was taken. Each pituitary was macerated in a centrifuge tube with a glass rod. To this was added 1.6 cc. of distilled water and one drop of 2 per cent NaOH. The material was then assayed in 24–25 day old female rats by injecting 0.2 cc. subcutaneously twice daily for 4 days and killing the rat 12 hours after the last injection. Ovarian and uterine weights were obtained at autopsy.

RESULTS

Daily food consumption of the thiourea fed rats is essentially normal during the first ten days of the experimental period and then decreases slightly below normal. Average food consumption per rat

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during the 20-day period was 342 grams when fed the stock diet and 304 grams when one percent thiourea was added. There was a tendency for the thiourea fed rats to scatter their food during the last ten days of the test period so that the decrease in food intake may actually be slightly greater than observed. Thiourea caused an average decrease of 42 grams in body weight, each animal losing weight during the experimental period. The control rats, on the other hand, uniformly gained weight.

As indicated in Table 1, thiourea causes an increase in total plasma protein due entirely to an increase in plasma globulin, the plasma

TABLE 1. INFLUENCE OF THIOUREA ON BLOOD PROTEIN LEVELS OF RATS

No. of rats	Treatment	Body weight start-end	Hemato-crit	Non-protein nitrogen	Total protein	Albumin	Globulin	a/g ratio
		gm.	per cent	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	
12	Thiourea	262–220	42.3±1.4[1]	65.7±2.7	6.94±0.15	3.83±0.21	3.10±0.20	1.30±0.14
17	None	236–251	45.3±0.6	49.8±0.6	6.34±0.07	3.81±0.09	2.53±0.10	1.56±0.08

[1] Mean deviation of mean $= \epsilon = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

albumin concentration remaining unaltered. Non-protein nitrogen is definitely higher in the plasma of the thiourea fed rats than in normal rats.

Enlargement of the thyroid glands was of anticipated magnitude whereas no effect on the weight of the testes, spleen, kidneys or liver was obtained. The pituitary was slightly heavier in the thiourea fed rats but the difference in weight is not significant. Other organs exhibited a decrease in weight namely, the adrenals, the seminal-vesicles and the ventral prostate. Since the body weight of the thiourea fed rats decreased during the experimental period, the organ weights computed on the basis of 100 grams body weight as well as the actual weights are presented (Table 2). The difference in adrenal weight becomes less striking when the body weight is considered, being 8.4 mg.

TABLE 2. INFLUENCE OF THIOUREA ON ORGAN WEIGHTS OF RATS

Treatment (no. of rats)	Ave. organ weight mg.		Ave. organ weight mg. per 100 gm. body wt.	
	Normal (15)	Thiourea (12)	Normal (15)	Thiourea (12)
pituitary	7.6	8.0	3.1	3.8
adrenal	27.5	18.4	10.0	8.4
thyroid	19.9	35.5	8.2	16.1
testes	2677.0	2993.0	1063.0	1172.0
seminal vesicles	853.0	378.0	377.0	170.0
ventral prostate	226.0	156.0	91.0	72.0
spleen	913.0	797.0	378.0	366.0
kidney	2227.0	2029.0	891.0	924.0
liver	9966.0	9397.0	3984.0	4249.0

(range 7.0–10.3) in the thiourea fed rats as compared with 10.0 mg. (range 7.2–12.3) in the controls. However, seminal vesicles were distinctly smaller in thiourea fed rats, being 378 mg. as compared with 853 mg. in control animals and this difference is not voided when the loss in body weight is considered.

An increase of the basophilic cells in the pituitary has been reported to occur in rats fed goiterogenic drugs. Any pronounced change in the cytology of this gland would presumably alter its activity or hormone content. Cytological studies were not undertaken but instead the gonadotrophic hormone content was estimated. Table 3 illustrates

TABLE 3. GONADOTROPIC POTENCY OF RAT PITUITARIES

Donors		Recipients			
Treatment	Amount A.P.	No. of rats	Average body wt.	Av. organ wt. ovaries	(mgs.) Uteri
	mgs.		gms.		
—	—	21	51.2	15.8(11–17)	28.6
none	7.6	14	58.4	59.4(44–81)	105.0
Thiourea	8.0	12	55.7	85.8(64–139)	64.0

that the gonadotrophic hormone potency of the pituitary is greater in the thiourea fed rats than that in a normal control.

A mild anemia was observed in some cases of thiourea feeding. Total erythrocyte counts ranged from 6.9 to 7.4 million cells in 5 of 8 animals studied whereas 10 normal rats averaged 8.6 million red cells. Total white count revealed 3 cases in which only 4–5 thousand cells were seen whereas the other rats were normal.

DISCUSSION

The maintenance of a normal albumin-globulin ratio in the rat is made possible, at least in part, by secretions of the endocrine glands. Following removal of the hypophysis, a decrease in serum albumin and an increase in serum globulin concentration is observed (Levin and Leathem, 1942; Levin, 1943). Similar changes are evident when the serum is analyzed by electrophoretic and by salt fractionation methods (Moore, Levin and Leathem, 1944). However, the increase in serum globulin in the hypophysectomized rat can be prevented by injecting thyroxin but the drop in albumin is not prevented. Thyroidectomy is followed by an increase in total serum proteins due entirely to the increase in serum globulin (Levin and Leathem, 1942). Administration of thiourea to induce a hypothyroid state should also result in blood protein changes simulating those following thyroidectomy and indeed an increase in total plasma protein is observed after 20 days of thiourea feeding which is due to an increase in plasma globulin since the plasma albumin concentration remained unchanged. Sloan and Shorr (1944) found the nitrogen balance became more positive in

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Graves' disease patients treated with thiouracil but no blood studies were reported. Williams and co-workers (1944 a) found total serum protein and NPN to be normal in thiouracil-treated patients but did observe increased urinary nitrogen retention. While the total serum protein values were within normal range it does not of necessity mean that there was no change. Furthermore, other clinical symptoms of the patients may obscure the results. Our data support the contention that thyroid activity is involved in the maintenance of plasma globulin but appears to exert little or no control over plasma albumin.

Although the rats lost weight during the 20-day experimental period the changes in the blood proteins do not simulate the changes observed during partial starvation. In this latter case a decrease in albumin as well as the increase in globulin is observed and the increase in globulin is less marked than that observed following thiourea feeding or thyroidectomy (Levin and Leathem, 1942). Furthermore, actual food intake is only slightly reduced by adding thiourea to the diet.

The weight of the spleen, liver and kidneys is unaltered by thiourea. Williams and co-workers (1944 b) previously reported that thiouracil is without effect on these organs. An effect on other organs by the goiterogenic drugs has been described. Kennedy and Purves (1941) observed an hypertrophy of the adrenal when Brassica seed was fed. Richter and Clisby (1942) observed no adrenal effect following phenyl thiourea and Williams and co-workers (1944 b) found the adrenals to be smaller following thiouracil but the size difference was due to a difference in body weights. The adrenals are definitely smaller in rats fed thiourea but the major portion of the weight difference is due to the difference in body weight. The goiterogenic drugs have no apparent effect upon the reproductive system since MacKenzie and MacKenzie (1943) observed normal estrous cycles and one instance of a normal litter. Furthermore, Williams and co-workers (1944 b) found testis weight unaltered by thiouracil when testis weight/body weight ratio was considered although the actual weight of the testes may be somewhat less than that of litter mate controls (Kennedy and Purves, 1941). The testes from rats in our series were unchanged in weight but seminal vesicle and ventral prostate weights were subnormal. The seminal vesicles were significantly below normal size suggesting a decreased production of androgen by the interstitial cells. Since several investigators have observed an increase in basophiles and a decrease in eosinophiles in the pituitary as result of drug administration (Griesbach, 1941; Kennedy, 1942; MacKenzie and MacKenzie, 1943) one might assume that a decrease in esoinophiles would reduce the amount of luteinizing hormone produced and a subsequent decrease in seminal vesicle weight would result as observed. At the same time the increase in basophiles in the pituitary would increase the follicle stimulating hormone and the gonadotrophic hor-

mone content of the gland should be greater. Assays of pituitaries from thiourea fed rats indicated that the gonadotrophic hormone content of these glands is somewhat greater than that of normal rats. The results of the ovarian stimulating capacity of the normal pituitaries agree with those previously reported (Reece and Weatherby, 1942; Reece and Leathem, 1944).

SUMMARY

A rise in total plasma protein concentration occurs in male rats fed one per cent thiourea for 20 to 22 days. This total protein rise is due entirely to an increase in plasma globulin, in that plasma albumin concentration does not change. Thus the blood protein changes simulate those observed after thyroidectomy.

Thiourea causes a loss in body weight although only a slight reduction in food intake occurs, but at the same time the anticipated increase in thyroid gland weight is observed. No significant change in the weight of the pituitary, testes, spleen, kidneys or liver is evident. However, a marked decrease in seminal vesicle weight is evident as well as a tendency for the adrenals and ventral prostate to be smaller.

Assay of the pituitary for gonadotrophic hormone reveals a greater hormone content in the glands from thiourea fed rats.

A mild anemia and/or leucopenia is observed in some cases.

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ESTROGEN ACTIVITY IN THE EVISCERATED RAT[1]

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THE EXTENSIVE work of Zondek (1934) and numerous other investigators offers strong evidence that the liver is largely responsible for the detoxification and removal from the blood of the natural estrogens. The spleen has also been implicated, and it is possible that other viscera may function similarly. However, it has been demonstrated by Heller (1940) and by Szego and Samuels (1943b) that other organs, especially uterus, may effect chemical conversions which increase the activity of the estrogens. Thus, these investigators were able to show that estrone can be converted to estradiol *in vitro* by fresh rat and rabbit uterus. Specific quantitative colorimetric methods were employed by Szego and Samuels (1943a) in the estimation of estrone and estradiol in the incubated solution.

It is well known that extradiol is much more active than estrone in stimulating an increase in uterine weight and, particularly, in uterine water content after parenteral administration. Since the uterus is apparently capable of converting the less active into the more active estrogen, at least *in vitro*, it appeared probable that the observed difference in activity *in vivo* might be due to a more rapid destruction of estrone, presumably by the liver and perhaps by other viscera.

In order to test the above hypothesis, the following series of experiments on estrogen activity in the eviscerated rat were carried out. However, no evidence for estrogenic activity in the eviscerated rat could be obtained, when the uterine water content of ovariectomized rats was used as an index.

METHODS

Young female rats of the Sprague-Dawley strain, weighing between 60 to 75 gm. were prepared for eventual evisceration by complete ligation of the vena cava above the entrance of the renal veins. They were then allowed to feed *ad libitum* on Purina fox chow for about a month. At this time, bilateral ovariectomy was performed by the dorsal approach. Two weeks later, the animals were fasted 24 hours and then either eviscerated or used as controls. The body weight of the fasted animals varied between 150 to 175 gm.

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[1] Read at the Twenty-Seventh Meeting of the Association for the Study of Internal Secretions, June 1944, Chicago, Ill.

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Abdominal evisceration was performed by the method of Reinecke (1943) which consists of hepatectomy and removal of the gastro-intestinal tract, leaving the kidneys intact. "Partially-eviscerated" animals were similarly treated, except that the liver was left *in situ*, deprived of its arterial circulation by a ligature about the coeliac and mesenteric arteries, tied prior to removal of the gut. Dehydration and hypoglycemia were circumvented in the eviscerated group by the subcutaneous administration of physiological saline and 20% glucose solutions. The superior viability of this type of preparation has been demonstrated (Reinecke, 1943). When an animal was to be carried for as long as 18 to 24 hours after evisceration, a glucose pellet was implanted subcutaneously.

Estrogen solutions were given subcutaneously in sesame oil or in physiological saline, intravenously in physiological saline, or *in utero* in 2% ethanol. In many instances, large doses of estrogen were administered by combined routes and in divided doses. Appropriate control solutions containing no estrogen were also used. Intravenous injections were made into the saphenous vein.

At the termination of each experiment, from 5 to 24 hours after the first injection, the animal was sacrificed and the uterine water content was determined by the method of Astwood (1938). This has been shown to be a reliable criterion of estrogenic activity in the immature rat. It proved satisfactory for our purposes in the adult ovariectomized rat. In the course of the experiments reported below, we found uterine weight, *per se*, too variable even in the untreated adult ovariectomized rat to be used as a basis for estrogen assay.

Where thyroid treatment is indicated, it refers to the incorporation of 1% dessicated thyroid in the diet beginning 6 days before evisceration. On the sixth or fasting day, the amount of thyroid powder given to each animal was the calculated average intake for the preceding 5 days. The estimated thyroid consumption was 67 mg. per 100 gm. body weight per day. Oxygen consumption rates of thyroid-treated animals, before and after complete or partial abdominal evisceration, were measured by the method of Shelley and Hemingway (cf. Roberts *et al.*, 1944).

RESULTS

It was first observed that subcutaneous doses of estradiol sufficient to cause large increases in uterine water content of intact ovariectomized rats had absolutely no effect on this index of estrogenic activity in eviscerated animals, otherwise similarly treated. Thus, 0.2 μg. of estradiol per 100 gm. body weight, administered subcutaneously in sesame oil and allowed to act for 10 hours before autopsy, caused a rise in uterine water content from a control value of 78.7% to a stimulated value of 83.5%, in the non-eviscerated preparation (Table I). No significant increase was noted in the eviscerated group.

To minimize such factors in the eviscerated rat as reduced circulation, poor absorption from subcutaneous sites, and lowered metabolism, which might conceivably interfere with the uterine water response to estrogen, a number of procedures were systematically employed. For example, estrogens were given intravenously, as well as

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subcutaneously. Large amounts were given in divided doses by combined routes and allowed to act over long periods of time. Topical administration to the uterus was also tried. Finally, rats whose metabolic rates were elevated by thyroid-treatment were used. A composite of the results obtained is given in Table I.

TABLE I. THE EFFECT OF EVISCERATION ON THE UTERINE WATER RESPONSE OF THE OVARIECTOMIZED RAT TO ESTRONE AND ESTRADIOL

	Total dose[1] (μg. per 100 gm. body wt.)	Route	Vehicle	Autopsy time in hrs.	% Uterine water		No. of Animals
					Ave.	Range	
I. *Non-Eviscerated*							
Estrone	0.2	subcu.	sesame oil	10	79.1	78.5–80.3	5
	0.2	iv.	saline	5	77.4	76.6–78.5	4
Estradiol	0.2	subcu.	sesame oil	10	83.5	81.7–84.2	5
	0.2	iv.	saline	5	83.5	82.5–84.4	4
	1.0	local	2% ethanol	6	81.7[2]	81.0–82.9	3
	2.5	iv.	saline	6	82.6[3]	82.4–82.8	2
Control	—	subcu.	sesame oil	10	78.7	77.9–79.7	4
	—	iv.	saline	5	77.0	75.8–78.2	4
	—	local	2% ethanol	6	77.0[2]	—	1
	—	iv.	saline	6	77.1[3]	77.0–77.2	2
II. *Eviscerated*							
Estrone	0.05	subcu.	sesame oil	6	79.6	78.8–80.4	2
	0.2	subcu.	sesame oil	6	78.5	78.4–78.5	2
	0.2	subcu.	sesame oil	10	79.1	78.0–79.7	5
	0.2	iv.	saline	5	78.0	77.7–78.3	4
	1.0	subcu.	sesame oil	6	79.9	79.8–79.9	2
	2.5 (1.5 + 1.0)	subcu. + iv.	saline + saline	10	78.1	75.2–80.2	4
	5.0 (1.5 + 2.5 + 1.0)	subcu. + subcu. + iv.	sesame oil + saline + saline	24	78.2	77.9–78.4	2
Estradiol	0.02	subcu.	sesame oil	6	79.1	78.0–80.2	2
	0.05	subcu.	sesame oil	6	78.8	77.3–80.3	2
	0.2	subcu.	sesame oil	6	80.4	80.3–80.5	2
	0.2	subcu.	sesame oil	10	79.6	77.9–80.8	5
	0.2	iv.	saline	5	78.4	76.0–80.0	4
	1.0	local	2% ethanol	6	77.4[2]	76.4–78.1	3
	2.0 (1.0 + 1.0)	subcu. + iv.	saline + saline	8	78.6	76.9–80.3	6
	2.5	iv.	saline	6	79.1[3]	77.8–80.2	3
	5.0 (1.5 + 2.5 + 1.0)	subcu. + subcu. + iv.	sesame oil + saline + saline	24	79.0	78.2–79.8	3
Control	—	subcu.	sesame oil	6	78.0	—	1
	—	subcu.	sesame oil	10	78.9	78.3–80.8	5
	—	iv.	saline	5	78.4	76.8–79.4	3
	—	subcu. + subcu. + iv.	sesame oil + saline + saline	18	78.2	—	1
	—	local	2% ethanol	6	78.2[2]	77.8–78.7	3
	—	iv.	saline	6	79.1[3]	79.0–79.2	2
III. *Partially-Eviscerated*							
Estradiol	0.2	iv.	saline	5	79.2	—	1
	1.0	iv.	saline	5	80.9	80.8–81.0	2
	2.5	iv.	saline	6	80.1[3]	79.4–80.7	2

[1] Divided doses, given by combined routes, are shown bracketed.
[2] Topical application to the uterus.
[3] Thyroid-treated animals.

Comparison of the different groups with respect to their percentage uterine water content at the time of autopsy reveals that only estradiol-treated non-eviscerated rats exhibited any significant degree of uterine stimulation. The rapid and good response by this group to intravenously administered estradiol is especially notable, since the effectiveness of estrogens given by this route has not been studied

in any detail. In the eviscerated group, however, even very large doses of estradiol (up to 5μg. per 100 gm.), given by combined subcutaneous and intravenous routes in divided doses, and allowed to act over periods as long as 24 hours after the first injection, were quite ineffective.

In the intact rat, the dosage of estrone equal to the effective dose of estradiol (0.2 μg. per 100 gm.) had no effect when administered under the same conditions. This, of course, is in agreement with the well-known observation that estradiol is much more active than es-

TABLE II. THE EFFECT OF EVISCERATION ON THE RATE OF OXYGEN CONSUMPTION OF THE THYROID-TREATED OVARIECTOMIZED RAT[1]

	Hours after injection of estradiol[2]		
	0	2.5	6
I. *Non-Eviscerated*	136	—	—
	160	—	—
II. *Eviscerated*	133	—	—
	167	—	—
	151[3]	125	—
	150[3]	84	—
	153	125	94
	111	101	65
III. *Partially-Eviscerated*	157	107	53
	120	121	80

[1] Figures in terms of cc. of oxygen consumed per 100 sq. cm. body surface per hr., at 27.5 degrees C.

[2] 2.5 μg. estradiol per 100 gm. body weight, injected intravenously in physiological saline.

[3] Saline controls, no estradiol.

trone. Like estradiol, estrone was completely ineffective in the eviscerated rat under a wide variety of experimental conditions.

In one series of experiments, estradiol was administered by injection through the cervix into the uterine lumen with a fine needle. One μg. per 100 gm. given in this way to the intact ovariectomized rat increased the average uterine water content from 77.0% in controls to 81.7%, 5 hours after application. No effect could be observed in similarly treated eviscerated animals (Table I, figures marked with the superscript 2).

Since Roberts *et al.* (1944) have previously shown that the metabolic rate of the eviscerated rat is reduced to one-fourth of its preoperative level within 2 hours after evisceration, an attempt was made to repeat some of the above studies in animals whose metabolic rate after operation was relatively high. This was accomplished by several days of thyroid feeding prior to operation. As Table II shows, the oxygen consumption of these animals was about 150 cc. per 100 sq. cm. of body surface per hour, before evisceration. It dropped rapidly after partial or complete evisceration, but remained relatively high and

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within the normal range (about 90 cc. O_2 per 100 sq. cm. per hr.) for a period of time sufficient to produce the uterine response in intact animals. Despite the relatively high metabolic rate after evisceration, estradiol in large doses (2.5 μg. per 100 gm. body weight, given intravenously and allowed to act for 6 hours) had no effect on the uterine water content of thyroid-treated eviscerated rats. This same dose administered to thyroid-treated intact animals had a marked effect (water content of stimulated uteri, 82.6%). These results are marked with the superscript 3 in Table I.

Finally, large doses of estradiol likewise had no clear-cut effect on the uterine water content of partially-eviscerated rats. Apparently this operation (consisting of removal of the gut only, leaving the liver intact but cut off from its arterial blood supply) was drastic enough to simulate the effects of complete evisceration.

DISCUSSION

The results obtained in the present study indicate that the natural estrogens estrone and estradiol are incapable of producing an increase in uterine water content in adult ovariectomized rats whose viscera have been removed. It is not possible to state at the present time whether or not other indices of estrogenic activity are similarly affected. The use of certain of these other indices, however, such as the vaginal smear technique, would be precluded by the necessarily short time available for assay in this preparation.

It appears highly unlikely that conditions of poor viability (reduced circulation, absorption, total metabolism) were alone responsible for these results. Thus, in the eviscerated animal, very large doses of estrone and estradiol were ineffective whether administered subcutaneously, intravenously, or in divided doses by combined routes, even when allowed to act for as long as 24 hours. Quite as ineffective was estradiol when applied topically to the uterus of the eviscerated preparation, and when administered in large doses to thyroid-treated eviscerated rats. In intact animals on the other hand, otherwise subjected to the same routine, large uterine responses were evoked.

It may be noted that the uterus did not undergo dehydration in the eviscerated rat within 24 hours after the operation. This fact is readily gleaned from Table I. The average per cent uterine water contents were as high in this group at the time of autopsy as in non-eviscerated controls. Grossly, circulation to the uterus appeared to be good in the eviscerated group.

The results reported demonstrate that removal of the viscera in some way blocks the action of estrogen in producing water imbibition by the uterus. Removal of the gut and tying off the arterial supply to the liver is apparently nearly or quite as effective in this regard. Two possible mechanisms for this phenomenon may be suggested. One

possibility is that the viscera function not only in inactivation or removal of estrogens from the blood, but are also essential for their chemical activation. Another likelihood is that the viscera furnish some metabolite essential for the uterine response.

Whatever the explanation for these results, the rôle of the viscera in the activity of the natural estrogens appears to be considerably more complex than previously supposed. The importance of these findings for studies on the metabolism of the estrogens is apparent.

SUMMARY

Estradiol and estrone were incapable of causing an increase in the uterine water content of the adult ovariectomized, eviscerated rat. This was found to be the case in animals treated with large doses of these natural estrogens (up to 5 μg. per 100 gm. body weight) administered subcutaneously, intravenously, or in divided doses by combined routes, and allowed to act for as long as 24 hours. This was likewise true when large doses of estradiol were given to animals whose total metabolism after evisceration was maintained by previous thyroid-feeding, or when applied topically to the uterus of the eviscerate. Non-eviscerated rats, otherwise similarly treated, showed large responses in uterine water content. It appears unlikely that poor viability was responsible for the refractoriness of the eviscerate preparation.

It is concluded that in the rat, the viscera are essential for estrogen activity as measured by the uterine water response.

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THE COMPARATIVE EFFECTS OF PROGESTERONE, ESTRADIOL, DIETHYLSTILBESTROL AND ITS MONOMETHYL ETHER ON THE EARLY BLASTOCYST OF THE RABBIT[1]

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THE SIZE of the 5 day rabbit blastocyst has been shown to be function of the amount of progesterone administered (Pincus & Werthessen, 1937). It was further demonstrated that the simultaneous injection of natural or synthetic estrogens profoundly altered the degree of response to a fixed dose of progesterone (Pincus & Werthessen, 1938). Thus, estradiol and estriol appeared to stimulate the growth of the blastocyst further, while estrone definitely inhibited it. The response of the endometrium differed from that of the blastocyst, in that estriol, for example, produced in the same rabbit a significant increment in blastocyst size and a notable diminution in endometrial response to the standard dose of progesterone, estradiol produced significant increments in both blastocyst size and endometrial growth within the lower concentrations of the dosage employed, while the higher concentrations resulted only in significant increases in blastocyst size; the endometrial response was essentially that of the controls.[3]

In the present study estradiol effects have been observed in a wide range of concentrations. Since the effects of estrone, in its benzoated form, appeared clear-cut in the previous study, its actions have not been reinvestigated. Diethyl stilbestrol was examined exhaustively because this compound is in common use therapeutically, and these aspects of its properties have not been studied to date. The monomethyl ether of diethylstilbestrol was likewise investigated thoroughly. As will be shown, it differed markedly in its actions from the parent compound.

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[1] Aided by a grant from Wallace & Tiernan, Inc., Belleville, N. J.

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[3] The accepted synergism between estrogens and progesterone has been demonstrated by the fact that prestimulation of the endometrium by estrogen leads to maximal progesterone effect per unit dosage.

The significance of this observation is not discussed or questioned in the present work. Since our observations are on uteri "normally" prestimulated by estrogen and deal only with the blastocyst, we felt that it could be neglected. The synergism and endometrial observations will be discussed in a later paper.

METHOD

Rabbit does were ovariectomized 16 to 24 hours after fertile copulation. All the animals then received 0.07 milligrams of progesterone subcutaneously twice daily for 3 days beginning the morning after ovariectomy or approximately 36 hours after copulation. Except for the control animals, varying doses of the estrogens were administered coincident with the second injection each day. The estrogens were given intraperitoneally so that differences in absorption rates between the estrogens, if any, would be minimized.

The animals were killed 18 to 24 hours after the last injection. The uteri were flushed with Lockes with dextrose added solution and the diameter of the blastocysts measured immediately. Representative sections of the uteri were fixed for measurement of the endometrial response. These latter data will be reported in a later paper.

RESULTS

The average egg size for each rabbit in the series and the average of the group at a particular dosage are given in the table (Table I). The average values for each dose were plotted against the logarithm of the dosage (Figure 1).

It is immediately apparent from the graphs that the three substances did not act in the same fashion. The differences are both qualitative and quantitative. The synthetic estrogens tend to inhibit the growth of the blastocysts as the dosage increases. Contrariwise, the natural estrogen tends to increase the size of the blastocysts.

The finding that the natural estrogen does not inhibit the growth of the blastocysts was quite unexpected. In our previous small series (Pincus & Werthessen, 1938) we had not obtained an inhibition of blastocyst growth with estradiol but had assumed that if a much higher dosage had been employed that inhibition would have occurred. This assumption was based on the fact that while neither estradiol nor estriol showed inhibition of egg growth at the dosages employed they appeared to inhibit proliferation of the endometrium in the earlier work without affecting the blastocysts. In this series, 297 gamma of estradiol failed to inhibit the growth of the blastocyst.

DISCUSSION

The fundamental premise of this work was the commonly accepted hypothesis that estrogenic substances are antagonistic to progesterone. This competition for effect on the vagina and edometrium has been frequently shown (Reynolds, 1939a). So too however has the need for sensitization of the uterus by estrogens prior to the assay of progesterone if maximum sensitivity in the test for progesterone was desired (Reynolds, 1939b). The data presented here suggest that the question of estrogenic hormone antagonism to progesterone and the physiological significance of this antagonism ought to be reexamined

The data presented here further indicate that more consideration must be paid to the type of natural estrogen with which a synthetic

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TABLE I. BLASTOCYST SIZE, EXPRESSED IN OCULAR MICROMETER UNITS, AS MEASURED AFTER BEING FLUSHED OUT OF UTERI 5 DAYS POST COITUS. ONE OCULAR MICROMETER UNIT WAS EQUAL TO 32.8 MICRONS.

Control—0.14 mg. progesterone per day for three days in 2 daily doses of 0.07 mgs. subcutaneously in oil.

Rabbit no.	Individual blastocyst sizes	Average per rabbit	Average per dose
134	17	17.0	
57	20	20.0	
62	22, 15, 16, 22	18.7	
69	37, 16, 19, 28, 23, 18	23.5	
73	32, 33, 27, 32, 21	29.0	
78	29, 28, 30, 29, 27, 28, 32	29.0	
127	16, 28, 29, 29, 29, 30, 30, 12, 11, 9, 13	21.5	23.9

All animals listed below received control dosage of progesterone

Total estrogen dosage ½ administered in single intraperitoneal injection daily for 3 days	Rabbit no.	Individual blastocyst sizes	Average per rabbit	Average per dose
		Estradiol		
0.84μg.	104	23, 18, 12, 8, 8	13.4	
	106	14, 11, 5, 9, 14, 11, 10, 9, 12, 14, 13	11.8	*12.5*
2.5μg.	107	12, 15, 13, 12, 13, 14, 15	13.4	
	109	21, 23	22.0	
	117	18, 18, 18, 20	18.5	
	115	25, 26, 18, 28, 27	25.0	*19.7*
5.0μg.	112	27, 21, 25	24.4	
	113	13, 19, 13, 11, 8, 13, 12	12.7	
	114	24, 28, 24, 16, 21, 20, 18, 16, 15, 21, 20, 21	20.3	
	118	15, 20, 17, 22, 14, 11, 16, 19	16.8	*18.5*
63μg.	132	30, 36, 40, 25, 19, 35, 28, 31	30.5	
	133	19, 23	21.0	*25.5*
180μg.	135	29, 28, 16, 13, 10, 14	18.3	
	138	20, 27, 21, 26, 24, 30	24.7	*21.5*
252μg.	140	33, 33	33.0	
	142	15, 13, 14	14.0	*23.5*
297μg.	144	17, 12, 11	13.3	
	145	34, 32, 33	33.0	*23.1*
		Stilbestrol		
210μg.	80	11, 10, 7	9.3	
	79	10, 11	10.5	
	24	10, 7	8.5	
	15	13, 8,10	10.3	*9.4*
105μg.	32	19, 13, 7, 21, 19, 8, 21	15.4	
	30	8, 11, 8, 8, 8	8.6	
	19	23, 26, 22, 17	22.0	*15.9*
52.5μg.	91	29, 26, 11, 10	17.4	
	83	26, 24, 25, 20	23.7	
	70	15	15.0	
	31	8, 8, 7, 7, 9	7.8	*15.9*

TABLE I. *Continued*

Total estrogen dosage ½ administered in single intraperitoneal injection daily for 3 days	Rabbit no.	Individual blastocyst sizes	Average per rabbit	Average per dose
21μg.	87	37, 32, 34, 28, 24, 38, 41, 38	34.0	
	86	16, 14, 13	14.3	
	75	36	36.0	*28.1*
5.3μg.	81	13, 11, 14, 15, 13	13.2	
	44	20, 20, 20, 8	15.6	*14.4*
2.6μg.	54	26, 30	28.0	
	53	16, 17, 16, 15, 16, 17, 17, 10	15.6	
	45	39, 44, 44, 39, 40, 49, 31	40.7	*28.1*
1.3μg.	63	15, 10, 9	11.3	
	59	12, 17, 17	15.3	
	48	18, 18, 18, 18, 18	18.0	*14.8*
0.65μg.	65	19, 17, 21, 21	19.5	
	64	30, 25, 24, 20	24.7	*22.1*
		Monomethyl ether of stilbestrol		
105μg.	17	14, 9, 12, 9	11.0	
		7, 6, 5	6.0	*8.5*
21μg.	23	11, 12, 11	11.3	
	18	18, 15, 19, 10, 15, 11	14.6	*12.9*
10.5μg.	25	13, 5, 11, 12, 7, 9, 12	8.4	
	128	14, 18, 17, 17, 13, 7, 13, 11, 13, 9, 10, 14, 16	13.2	
	130	17, 16, 20, 15, 20, 15, 20, 22, 13, 17, 18	17.6	*13.1*
5.3μg.	33	36, 34, 18, 22, 14, 18, 22, 13, 14, 15	20.6	
	47	20	20.0	
	26	18, 25, 29, 22, 24	23.6	*21.4*
2.6μg.	40	25, 28, 28	27.0	
	27	8, 8, 11, 2, 8	9.4	
	41	26, 29, 10, 28, 21, 23, 23, 29	23.7	*20.0*
1.3μg.	39	12, 11, 21, 27	17.7	
	37	24, 17, 26, 17	21.0	
	36	17, 13, 15, 19, 28, 12, 10, 8, 14	14.6	*17.8*
0.65μg.	58	15, 12, 7, 15	12.8	
	51	19, 17, 18	28.0	
	50	35, 20	27.5	
	49	23	23.0	*18.6*
		Estradiol benzoate		
2.5μg.	121	22	22.0	
	123	13, 26, 15, 14, 12, 10, 14, 9	14.1	*18.0*
		Unoperated controls		
	8	47, 38, 44, 49, 39	43.4	
	9	45, 44, 44, 44, 38, 38, 44, 44, 41	42.4	*42.9*

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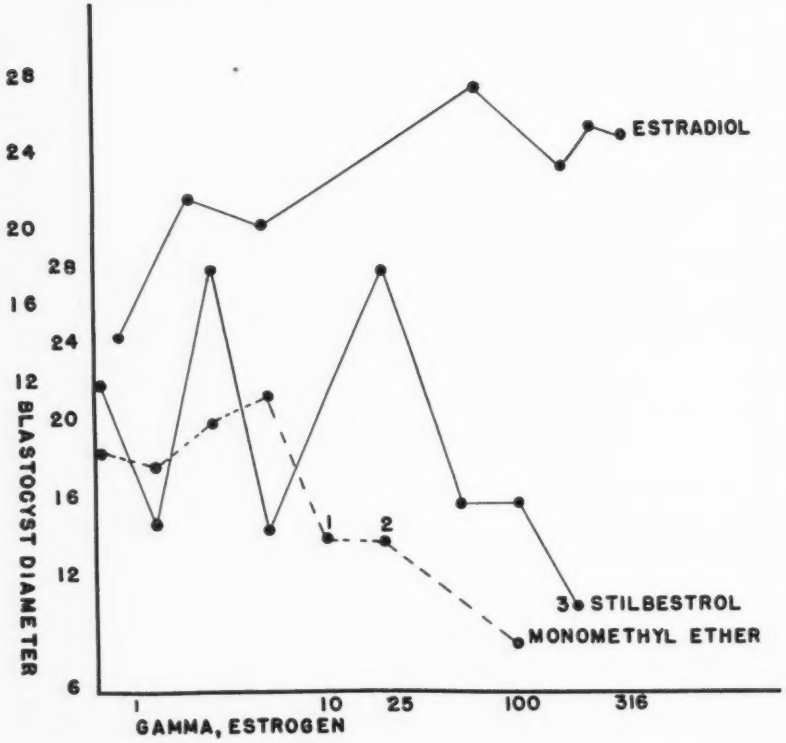

FIG. 1. The figure presents graphically the data presented in Table I. The dosage scale is logarithmic. The blastocyst size scale is numerical. The curve for Estradiol has been elevated above those for the synthetic substances for clarity, although the grid values are the same. The curves are identified by the appropriate name of the substance at the end of each curve.

The numbers (1, 2, 3) at certain points in the figure indicate the following:

1) Probability that this point is significantly different from the control level is less than .05 and greater than .01.

2) The probability values are less than .05 and greater than .01.

A grouping of points 1 and 2 as a single value gives a probability value of less than .01.

3) The probability value of this point is less than .01.

substance is being compared. If it is assumed, as it is generally believed, that estradiol is the true ovarian secretion, then it follows that while estrone and synthetic substances such as those examined here behave in general in similar fashion; this does not mean that any of these substances are truly capable of substituting completely for the natural estrogen, certainly not in the early pre-implantation stage of pregnancy.

Variance and Cause of Deviations of Effect

Examination of the variance within the data indicates strongly that further undisclosed differences exist between the 3 substances studied. It is obvious in figure 1 that there is a great deal more scatter between the points for stilbestrol than for the other 2 substances. The variance has been studied from the statistical viewpoint and, while not revealing any further information of use at present, confirmed the impression that there was a real difference between the variance encountered in the stilbestrol and its monomethyl ether series.

Since care had been taken to study both substances at the same time, with the same group of animals and under identical conditions, the significant differance must at present be ascribed to the substance employed. The simplest explanation would appear to be that monomethyl ether by virtue of a slower degradation rate provided a smoother effect than did the stilbestrol.

The inhibition of blastocyst growth by the synthetic substances and the apparent lack of this stimulation by the native estrogen require explanation.

The route of administration could not have been a contributing factor, in our opinion. The intraperitoneal route was chosen to reduce the effect that differences in rate of absorption might have played had the subcutaneous route been used. Intravenous injection would have been the method of choice had it not been requisite to use olive oil as the carrier of the compounds. Some of the material injected by the intraperitoneal route may have entered the portal circulation through absorption into the mesenteric veins. It has appeared most reasonable to assume however that the major portion of the injected material entered the lymphatic drainage of the peritoneal cavity and was thus in a sense a "slow" intravenous injection via the jugular. We employed estradiol benzoate in 2 animals at the 2.5 gamma dosage level injected intravenously to test the hypothesis in part. As shown in the table there is no real difference in egg size between these animals and those receiving an equivalent dosage. Whereas, had hepatic degradation been a significant factor, this dosage should have shown it (*vide* efficacy of 10 gamma of the monomethyl ether).

The divergence in the present findings for estradiol from those reported earlier requires comment. In the earlier work a significant increment in blastocyst size was noted at the 3 and 75 gamma dosage levels. No such increment was noted here. The most reasonable explanation would appear to be that the control egg size in this group was larger than in the previous study. Our average for this study was 700 microns—in the earlier work it was 500 microns. The increment in egg size noted must be due to either animal differences or some differences in progesterone, although in both studies crystalline progesterone was used.

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Furthermore—as can be seen from the unoperated control egg size—there was little room for an increment which would have been statistically significant. In this sense the choice of progesterone dosage in this work was unfortunate. It was used throughout however even when it was noted that the egg size was close to maximum expected growth because it was felt that a decrement in size was the most probable end result of estradiol administration.

Bearing in mind the earlier findings of estradiol stimulation of egg growth and the apparent increase in egg size with increasing dosage of estradiol seen in this study,[4] the conclusion was inescapable that estradiol injected intraperitoneally if not a stimulant to early blastocyst growth, certainly is not an inhibitor.

Yet estrone, stilbestrol and its monomethyl ether inhibited blastocyst growth when administered in the same fashion. The inhibitions observed are not identical quantitatively either in "potency" per gamma of estrogen injected nor are—judging from differences in variance—qualitatively the same.

If future work supports the observations cited here then it follows that a significant faculty of estradiol is its noninterference with progesterone in the early stages of pregnancy. This property did not appear to be shared by estrone, stilbestrol and its monomethyl ether. Judging by earlier work estriol and 5-methoxy-9-ethoxy-1′, 3′-diketo-1, 2 cyclopentenophenanthrene may share it in part (Pincus & Werthessen, 1938).

It is further noteworthy that in these data the monomethyl ether of stilbestrol is more potent as an inhibitor of egg growth than stilbestrol. It must therefore have a greater effect on the uterus, since its effect on the blastocysts could only be mediated through the uterus.

It has been shown (Geschikter & Byrnes, 1942) that stilbestrol is more potent in inducing a vaginal reaction than the monomethyl ether. The ratio is now considered to be about three to one in favor of the parent substance when orally administered. In this study, however, with due regard for the variation in egg size encountered the parent substance exhibited no more than one-fourth the potency of the monomethyl ether in inhibiting the growth of the blastocyst. This may or may not have been due to a longer period of activity in which the monomethyl ether could presumably have functioned.

We must therefore assume for the synthetic compounds a disproportion in their activities as gauged by various modes of bioassay. Certainly the data presented indicate for the monomethyl ether of stilbestrol a greater utero-tropic faculty than that possessed by stilbestrol.

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[4] The increment in size observed may be more apparent than real since it did not extend beyond statistically significant deviations from the controls.

SUMMARY AND CONCLUSIONS

Estradiol, diethylstilbestrol, and the monomethyl ether of diethylstilbestrol have been tested for their effects on the growing blastocyst of the ovariectomized rabbit given 0.14 mg. of progesterone daily. Estradiol was found to tend toward stimulating blastocyst growth, while the synthetic estrogens were definitely able to inhibit such growth. The monomethyl ether of diethylstilbestrol was found to be 4 times as potent as the parent substance in its effect on the uterus. This stimulant effect upon the uterus was produced with significantly less variance, probably the result of the previously observed prolonged and more constant estrogenic effect of this compound.

The evidence presented in this study indicates that the synthetic estrogens are not complete substitutes for estradiol from a qualitative viewpoint. Since they are qualitatively different in effect, quantitative comparison by this mode of assay is impossible.

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THE CAUSE OF ABNORMAL RETENTION OF INGESTED WATER IN ADRENALECTOMIZED RATS

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IT IS WELL RECOGNIZED that there is a failure of normal diuresis after the ingestion of large amounts of water in adrenalectomized animals as well as in Addisonian patients. As a result of the report of Robinson, Power and Kepler (1941), the "water test" has become well established as a useful laboratory adjunct in the diagnosis of Addison's disease.

The mechanism of the failure of diuresis in the presence of adrenal insufficiency has not been extensively studied. One might be inclined to localize the defect in the gastro-intestinal tract inasmuch as intestinal absorption is known to be impaired in the adrenalectomized animal. That the unexcreted water is retained chiefly in the gut would seem unlikely, however, for it has been shown that the retention is accompanied by signs of water intoxication (Rigler, 1935; Swingle *et al.*, 1937; Gaunt *et al.*, 1937). It would appear, therefore, that excess water actually reaches the tissues. Water could conceivably be attracted to the tissues and held there by an abnormal affinity on the part of the cells. It has been shown, in fact, that excessive cellular hydration may accompany adrenal insufficiency (Harrop, 1936; Darrow *et al.*, 1939). Another possible explanation of the water retention might be that renal excretion is defective. The following experiments were performed in order to evaluate the contribution of each of these possible mechanisms in the production of the phenomenon.

METHODS

Male rats of Sprague Dawley strain, weighing between 150 and 200 gms., were used. Bilateral adrenalectomy was performed 48 hours before the administration of water. No extra salt or hormone was given. With the exception of some of the animals in the series used for the study of absorption (Table I), the rats were allowed access to food and water until the experiments were started. Controls and operated animals were run simultaneously. The rats were placed in metabolism cages after which 5 cc. of water per 100

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[1] With the technical assistance of Ethel Buchwald.

gm. of weight was administered by stomach tube at hourly intervals until 4 doses had been given. One hour after the last dose, the animals were weighed and injected intraperitoneally with an anesthetic dose of nembutal. Fifteen minutes later, the lateral tail vein was nicked and a sample of freely flowing blood taken for hematocrit determination by the method of Van Allen (1925). Sodium thiocyanate solution (0.5 cc. containing 3 mg.) was carefully injected intravenously by way of a tail vein. One-half hour later, the animal was exsanguinated by direct incision of the abdominal aorta. After removal of the proteins with trichloracetic acid, the thiocyanate content of the serum was determined by means of the iron color-reaction. Readings were made in a spectrophotelometer. Bladder urine was aspirated and measured. The contents of the stomach and intestine were stripped from the organs and weighed. Unabsorbed water was estimated by subtracting the average weight of contents in rats receiving no water from the average in rats given water. Carcasses were weighed and dried to constant weight at 110° C.

Nephrectomized rats were treated the same as those above, except that water was administered immediately after the operation, and the total dose was only 15 cc. per 100 gm. A reduced dose was chosen in order to give a total retention comparable to that of adrenalectomized animals.

RESULTS

Absorption by G-I Tract: It was quite obvious that the major deficiency did not consist of failure of absorption (Table I). In the case

TABLE I. THE EFFECT OF ADRENALECTOMY ON THE ABSORPTION OF WATER BY THE G-I TRACT

		Group given water	Group given no water	Difference (Retention of water)	Per cent of dose retained	Per cent of water absorbed
Fasted	Adrenalectomized	Stom. 0.9±0.3 Intes. 2.7±0.4	0.3±0.1 1.5±0.3	0.6±0.3 1.2±0.5	3% 6%	91%
	Normal	Stom. 0.5±0.2 Intes. 2.1±0.4	0.1±0.1 1.6±0.2	0.4±0.2 0.5±0.5	2% 3%	95%
Fed	Adrenalectomized	Stom. 3.4±0.5 Intes. 3.5±0.3	1.0±0.2 2.7±0.1	2.4±0.6 0.8±0.3	12% 4%	84%
	Normal	Stom. 1.9±0.3 Intes. 3.5±0.2	0.8±0.1 3.5±0.2	1.1±0.3 0.0±0.3	5% 0%	95%

In the first three columns, the averages with their standard errors represent grams or cc. of fluid per100 gm. of rat. Each group of fasted rats contained 4 rats, fed groups 8 rats. The fast was 18 hours in duration.

of fasted rats, the adrenalectomized group absorbed 91% of the water as compared to 95% for the intact group. Unabsorbed water in the intestine in the fasted adrenalectomized animals amounted to 6% as against 3% for normal animals. Thus only 3% of the administered water remaining unabsorbed in the gut and 4% in the entire digestive tract could be attributed to adrenal insufficiency.

Fed adrenalectomized rats showed a greater tendency to retain water in the G-I tract than did fasted animals. The proportion of un-

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absorbed water recovered from this source in operated rats was significantly greater than normal (16% versus 5%). It may be seen from Table I, however, that this was not due principally to stasis in the intestine but rather to a sluggish gastric motility inasmuch as the bulk of the water was recovered from the stomach.

Even though a significant amount of water failed to traverse the wall of the gut in the group of fed adrenalectomized rats, the actual total retention in the G-I tract accounted for only 25% of the observed gain in weight (Table II). The amount of water which actually

TABLE II. THE RETENTION OF WATER IN ADRENALECTOMIZED, NORMAL AND NEPHRECTOMIZED RATS (FED)

		Urine excreted		Total water remained in animal (wt. gain in gms.)		Total water retained in G-I tract	Water absorbed	% water in carcass		Expected % water in carcass
		No.		No.				No.		
Adrenalectomized rats	Water	17	3.7±0.7	17	12.9±0.7	3.2±0.5	9.7±0.8	17	70.9±0.4	71.4
	No water							18	68.6±0.4	
Normal rats	Water	10	12.6±1.0	10	3.0±0.8	1.1±0.3	1.9±0.8	10	68.3±0.5	
	No water							11	68.3±0.6	
Nephrectomized rats	Water			22	13.4±0.4	2.5±0.5	10.9±0.6	21	70.7±0.4	71.4

Values in the first 4 columns represent gms. or cc. of water per 100 gm. of rat.

reached the tissues was 9.7 cc. per 100 gm. out of a total 12.9 cc. retained in the animal. A calculation of the expected increase in water concentration of the carcass gave a value which was somewhat higher but fairly close to that which was observed. It should be noted that this calculation was made with the assumption that the extra water was equally distributed throughout the body. Actually, it would be expected that the per cent increase in the carcass, which contains bone, should be less than in the viscera which are composed entirely of soft tissue.

Thiocyanate Volume: One may assume for practical purposes that injected thiocyanate is distributed almost exclusively in the extracellular compartment. It is at least agreed that fluctuations in thiocyanate volume run parallel to changes in extracellular fluid volume (Kaltreider *et al.*, 1941; Winkler *et al.*, 1943). Changes in extracellular fluid in the experimental animals are recorded in Table III. Adrenalectomized rats which received water showed a distinct rise in extracellular fluid volume. The increase amounted to 9 cc. per 100 gm. of rat. Inasmuch as the total retention of absorbed water in this group was 9.7 cc., it is apparent that the great bulk of fluid was held in the extracellular compartment.

Nephrectomized rats, as would be expected, showed a similar increase in extracellular water, although the increment was actually not so large as that observed in adrenalectomized rats. This difference

between the nephrectomized and adrenalectomized animals cannot be considered real, however, because of the magnitude of the errors of estimate. The important conclusion which may be drawn is that the effect of adrenalectomy was similar to that of nephrectomy. In both instances, the preponderance of retained water was held in tissue spaces rather than in cells.

Hematocrit and Hemoglobin Concentration: In an acute experiment, it is possible to detect changes in plasma volume by measurements of hematocrit and hemoglobin concentration. In Table III, it is seen that

TABLE III. THE DISTRIBUTION OF ABSORBED WATER

		Thiocyanate Volume—cc.		Extracellular retained water-cc.	Intracellular retained water-cc. (by difference)	Hematocrit	
		No.				No.	
Adrenalectomized rats	Water	12	41 ±1.1	9 ±2.2	0.7	19	43 ±0.9
	No water	10	32 ±1.9			23	41 ±1.0
Normal rats	Water	8	36 ±1.7	2		10	38 ±1.0
	No Water	10	34 ±1.3			13	41 ±0.9
Nephrectomized rats	Water	11	41 ±0.9	7 ±1.6	4.4	19	31 ±1.1

the average hematocrit values are higher in adrenalectomized rats which received water than in the group given no water. Unfortunately the error of the difference is too great to conclude that this small rise in hematocrit is significant. In order to circumvent the errors inherent in a statistical method, blood samples were taken both before and after water ingestion in a small series of rats (Table IV). In every instance, there was a rise in both hematocrit and hemoglobin. It is therefore fair to conclude that plasma volume contracted.

In nephrectomized rats, the findings were quite different. The marked drop in hematocrit after water ingestion indicated a rise in plasma volume (Table III).

DISCUSSION

The results indicate that the failure of water diuresis encountered in a series of adrenalectomized rats was not due principally to stasis of water in the G-I tract. A significant retention of water in the alimentary system was encountered in fed rats, but its presence was due largely to poor gastric motility. Although more water remained unabsorbed in the intestine of both fed and fasted adrenalectomized animals than in normal animals, and it be granted that absorption was measurably impaired, nevertheless the magnitude of the retention was small in comparison with the total water retained by the animal. These observations differ somewhat from those of Gaunt (1944) who has recently made an extensive study of water intoxica-

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tion in the rat. He found in adrenalectomized animals that, although a certain proportion of orally administered water accumulated in the tissues, unabsorbed water comprised a very substantial component of that which remained in the animal. It is quite possible that the discrepancy between Gaunt's observations and our own may be attributable to a difference in the condition of the animals. In the present experiments, it was noted that gastro-intestinal stasis was most marked in rats which seemed to be in poor condition at the end of the experiment.

The accumulation of excess fluid in the extracellular compartment points toward a renal aberration as the major cause of the water retention encountered in adrenalectomized rats. The renal abnormality

TABLE IV. THE HEMATOCRIT AND HEMOGLOBIN IN ADRENALECTOMIZED RATS RECEIVING WATER

Rat No.	Hematocrit		Difference	% Increase	Hemoglobin gms. per 100 cc.		Difference	% Increase
	Before	After			Before	After		
1	41	43	+2	5	19.5	20.1	+0.6	3
2	42	47	+5	12	20.7	21.7	+1.0	5
3	40	42	+2	5	19.1	19.9	+0.8	4
4	42	45	+3	7	21.1	21.3	+0.2	1
5	38	40	+2	5	19.0	21.3	+2.3	12
6	—	—	—	—	17.8	20.3	+2.5	14
				Aver. 6.8%				Aver. 6.5%

Hemoglobin was determined by conversion of the pigment to cyanmethemoglobin. Readings were made on a spectrophotelometer.

cannot be ascribed to hypotension inasmuch as a fall in blood pressure is not expected to occur (Gaunt 1944). It would thus appear that in the presence of excessive hydration, tubular reabsorption of water is not inhibited in the normal manner. Gersh and Grollman (1939) have found that there is an increased tubular reabsorption of water in the rat in the presence of adrenal insufficiency. It is possible that the increased water excretion which occurs after the administration of adrenal cortical compounds is due to an opposite behavior of the tubule cells wherein water reabsorption is decreased below normal (Silvette and Britton, 1938; Ragan *et al.*, 1940; Mulinos *et al.*, 1942). There is, of course, excellent evidence that tubular reabsorption of various electrolytes is influenced by adrenal cortical hormone (Harrison and Darrow, 1939).

The rise in hematocrit in adrenalectomized rats after excessive water ingestion is of considerable interest. A similar observation was made by Gaunt (1944). He found, however, that hemoglobin did not always rise in proportion to the hematocrit. He also observed a tendency to excessive hemolysis in the drawn blood and concluded, therefore, that the rise in hematocrit was probably due in part to swelling

of the red cells. In the present experiments, a tendency to excessive hemolysis was not encountered and hemoglobin concentration was found to rise concomitantly with hematocrit. It seems unlikely that a splenic discharge of red cells could be of sufficient magnitude to counteract the diluting effect of extracellular hydration. The suspicion is therefore justified that there was a true reduction in plasma volume. One would expect the reverse to occur, namely, a rise in plasma volume accompanying a rise in total extracellular fluids, as was the case with nephrectomized animals. The discrepancy might be explained on the assumption that with water intoxication in the adrenalectomized animals, there is an increase in capillary permeability with loss of plasma through the vessel walls. Gaunt's observation (1944) that there is a decline in plasma protein concentration under such circumstances is consistent with such a course of events. A decline in plasma protein would promote a decrease in plasma volume. Such a shift of protein into tissue spaces would contribute to the formation of interstitial edema but it seems unlikely that such a shift can constitute the sole mechanism responsible for the failure of diuresis.

SUMMARY

Although there is a demonstrable delay in the intestinal absorption of water in adrenalectomized rats and a decreased emptying time of the stomach, these are only minor causes of failure of excretion when water is ingested in large quantities. The greatest portion of the administered water is retained in the extracellular compartment and probably remains unexcreted because of failure of elimination by the kidney. Plasma volume (as reflected by hematocrit) is slightly decreased under such circumstances. It is suggested that the low plasma volume is due to leakage of protein through the capillaries.

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THE INNERVATION OF THE AVIAN HYPOPHYSIS

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INTRODUCTION

NUMEROUS investigations have indicated that certain visual stimuli modify the gonadotropic functions of the anterior lobe of the avian hypophysis. It has often been observed that regularly increased periods of daily illumination activates the dormant avian gonads during periods when they are normally quiescent. Excellent reviews of the literature regarding the effects of light upon birds are those of Bissonnette (1938) and of Brooks (1940).

Most investigators have concluded that the eyes are the sole receptors for the light stimuli and have reasoned that nerve impulses pass by way of the optic nerves and hypothalamus to influence the pars distalis of the hypophysis. However, few reports concerning the innervation of the avian pituitary gland are in the literature.

Considerable disagreement exists as to the question of whether nerve fibers of the hypothalamico-hypophyseal system innervate the pars distalis of any species. For a study concerning this problem, the bird hypophysis is particularly suitable in that the pars distalis is completely separated from the pars nervosa except for a small strand of the pars tuberalis which extends from the pars distalis to envelop the infundibular stalk.

This study was undertaken to ascertain: first, the innervation of the avian hypophysis; and second, to determine whether nerve fibers pass from the hypothalamico-hypophyseal nerve bundle to the anterior lobe of a species in which the processes infundibuli is separated from the pars distalis.

MATERIALS AND METHODS

Pyridine-silver preparations were made of 10 adult chicken hypophyses. Immediately after each bird was killed the top of the cranium was removed to permit quick penetration of the fixing fluid. This consisted of absolute alcohol with 6 drops of ammonium hydroxide per 100 cc. Later the heads were trimmed to include only the lower half of the brain, the base of the skull with the sella turcica, and the hypophysis with adjacent structures. A 2% nitric acid solution was used as a decalcifying agent. The tissues were then pre-

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pared according to the Larsell modification of the pyridine-silver method (Rasmussen, 1938), embedded in paraffin and sectioned serially.

OBSERVATIONS

Pyridine-silver preparations reveal a conspicuous bundle of nerve fibers descending in the lower part of each lateral wall of the third ventricle (figs. 1 and 2). Such fibers of the two sides converge in the floor of the ventricle and pass posteriorly in the hypophyseal stalk (fig. 2). A number of these fibers arise from a nucleus of large cells situated in the wall of the hypothalamic portion of the third ventricle. Another group of fibers can be followed from an anterior region neighboring the upper portion of the optic chiasma (fig. 1). The exact origin

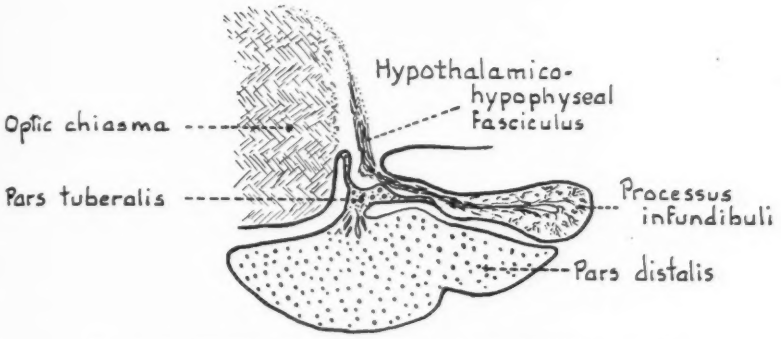

FIG. 1. A composite drawing constructed from sagittal sections of the chicken hypophysis.

of this last group of fibers could not be ascertained from pyridine-silver preparations.

The hypothalamico-hypophyseal fasciculus after its formation in the floor of the third ventricle passes by way of the stalk to terminate in the pars nervosa (fig. 1). In the fowl the pars nervosa is convoluted and contains a cavity which is continuous with the third ventricle. The proportion of glandular parenchyma to the amount of nervous tissue is much greater in the pars nervosa of the bird than in the mammalian hypophysis. The nerve fibers of the hypophyseal fasciculus retain a central position and small tufts of fibers branch from the main fiber bundle to ramify into each of the succular convolutions.

From the hypophyseal fasciculus a few small nerve fibers, which resemble collaterals, pass into the tissue of the pars tuberalis. These fibers can never be followed far into the glandular substance. Other fibers, autonomic in origin, accompany vascular channels into this part of the pituitary gland. In general there are relatively few fibers of either origin in this division of the chicken hypophysis.

In the region where the pars tuberalis contacts the pars distalis,

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there is a considerable number of thin walled vascular channels which are continuous with the sinusoids of the anterior lobe (fig. 5). However, no nerve fibers are associated with or accompany these channels into the pars distalis. Although the arteries adjacent to the hypophysis are accompanied by prominent nerve bundles (fig. 3) which often exhibit small ganglionic masses along their course (fig. 4), an examina-

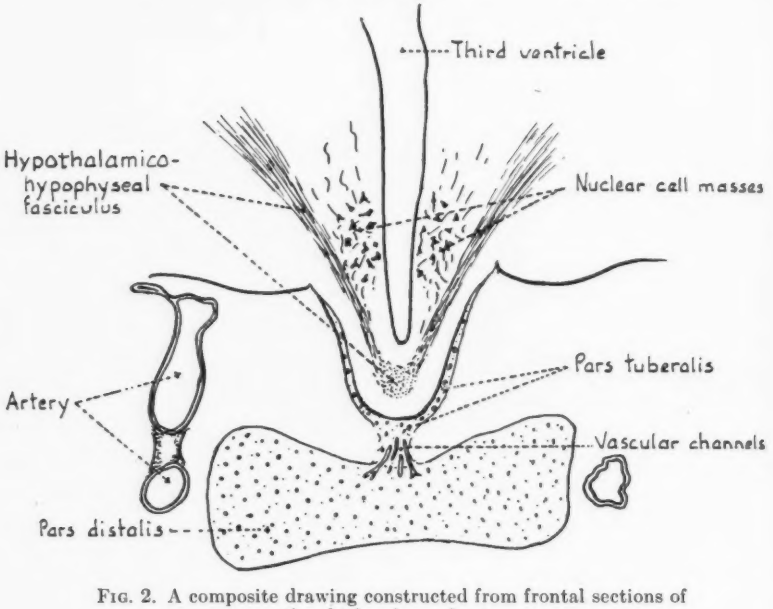

FIG. 2. A composite drawing constructed from frontal sections of the chicken hypophysis.

tion of the anterior lobe revealed no nerves entering the glandular tissue from the capsular region.

DISCUSSION

Providing that nerve connections exist between the hypothalamus and the pars distalis (retino-anterior pituitary reflex) as has been

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PLATE I

FIG. 3. A frontal section of the hypophyseal region of the chicken pituitary gland showing nerve fibers (N) and ganglionic cells (G) accompanying an artery (A) adjacent to the pars distalis (Pd). The pars tuberalis (Pt) extends from the pars distalis to the hypophyseal stalk (Hs) (×55).

FIG. 4. The ganglionic cells that accompany the internal carotid artery at a higher magnification (×800). The relation of these cells to the nerve fibers and artery is shown in figure 3.

FIG. 5. A frontal section showing the continuation of sinusoids (S) between the parts tuberalis (Pt) and the pars distalis (Pd) (×100).

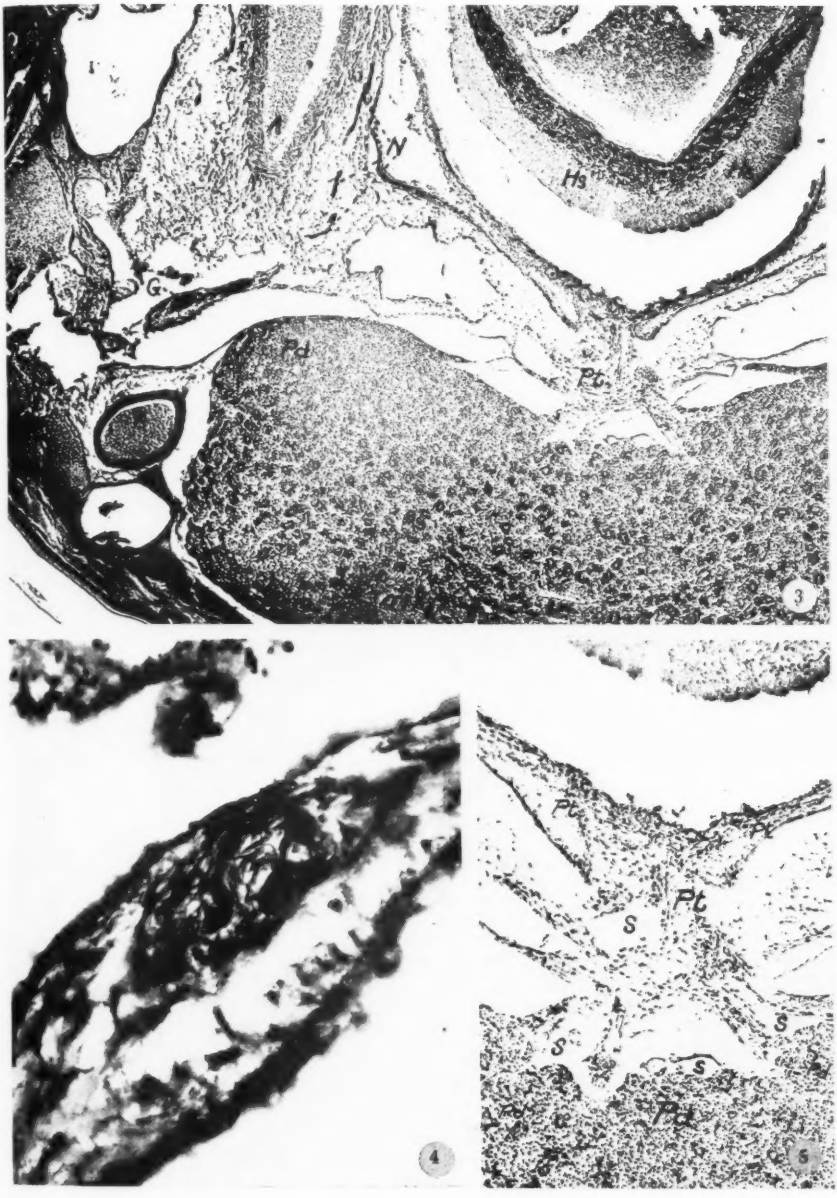

PLATE 1 (See opposite page for description.)

proposed by investigators concerned with the effect of illumination on the avian gonad, one would expect to find these fibers passing from the hypophyseal stalk through the pars tuberalis to the pars distalis. The few fibers that do pass from the infundibular bundle to the pars tuberalis never reach the anterior lobe. In mammalian species in which there are autonomic nerve fibers in the pars distalis, the fibers for the greater length of their course are associated with fairly well defined arteries (Rasmussen, 1938; Hare, 1938). Of interest is the absence of well defined areteries in this lobe of the chicken hypophysis. This may also account for the absence of the autonomic nerve fibers in the pars distalis of the bird. Considering the specificity in which nerve fibers were demonstrated in the various structures adjacent to the pars distalis, it is doubtful that the failure to find nerve fibers in the anterior lobe can be attributed to faulty impregnation of the tissue.

The fact that nerve fibers of the hypothalamico-hypophyseal system pass to the pars tuberalis and the processus infundibuli suggests a functional relationship between the hypothalamus and these two divisions of the bird hypophysis. A comparable relationship cannot be proposed between the hypothalamus and the pars distalis, at least on the basis of demonstrable nervous connections.

However, a relationship may exist between the pars tuberalis and the pars distalis through the prominent vascular channels. It is conceivable that secretions of other portions of the pituitary gland or from the hypothalamus may in some way influence the secretory activity of th · anterior lobe. Recently, Scharrer and Scharrer (1940) and Paley (1943) and others have placed considerable emphasis on the neurosecretory cells of the hypothalamic nuclei. Such cells may be found to play a greater role in the endocrine regulation than at present believed. Certain mammalian experiments indicate a humoral link between the nervous system and the anterior lobe of the pituitary gland. Keller and Hamilton (1937) Dandy (1940) and others, have observed normal sex functions following the section of the hypophyseal stalk of the dog and human. On the other hand, hypothalamic lesions usually result in abnormal sex cycles and many investigators contend that the hypophyseal stalk must be intact for normal functioning of the pars distalis.

SUMMARY

Considerable disagreement exists as to the question of whether nerve fibers of the hypothalamico-hypophyseal fasciculus innervate the pars distalis of any species. The bird hypophysis is particularly suitable for such study in that the pars distalis is completely separated from the processus infundibuli except for a small strand of the pars tuberalis which extends from the pars distalis to envelope the infundibular stalk.

Silver preparations of the diencephalon and hypophysis reveal a prominent hypothalamico-hypophyseal fasciculus. Fibers of this fasciculus, as nearly as can be traced in pyridine-silver preparations, can be followed from a region dorsal to the optic chiasma and from a nucleus in the lower wall of the third ventricle. Fibers of other origins may be contained in the hypothalamico-hypophyseal system.

The majority of the hypothalamico-hypophyseal fibers terminate in the processus infundibuli; however, some fibers pass into the pars tuberalis. Other fibers not contained in the hypothalamico-hypophyseal fasciculus and probably of autonomic origin, accompany vascular channels into the pars tuberalis but do not pass into the pars distalis.

No nerve fibers could be traced from the hypothalamico-hypophyseal fasciculus, through the pars tuberalis into the pars distalis. Arteries adjacent to the hypophysis are accompanied by prominent nerve bundles which often possess small ganglionic masses along their course. Nerve fibers of this type could not be traced into the pars distalis.

ACKNOWLEDGMENT

I wish to express my appreciation to Dr. A. T. Rasmussen for helpful suggestions and criticism.

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THE EFFECTS OF BILATERAL CERVICAL SYMPATHECTOMY ON THYROID ACTIVITY

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THIS INVESTIGATION was undertaken to obtain further evidence regarding the functional innervation of the thyroid gland. We were primarily interested in ascertaining whether bilateral cervical sympathectomy in guinea pigs altered the metabolic response to thyrotropic hormone and to exposure to cold.

The effect of bilateral sympathectomy on the basal metabolic rate of cats and rabbits with a review of the pertinent literature has been presented previously (Brock, Doty, Krasno and Ivy, 1940).

It appears to be established that thyrotropic hormone stimulates hyperplasia of the thyroid when devoid of a nerve supply, as in the case of autotransplants (Krayer, 1933; Eitel, 1934; Solomon and Severinghaus, 1936; Marine and Rosen, 1939; Manley and Marine, 1915) and of tissue culture (Canzanelli, 1938). However, it has been reported that the thyroid of sympathectomized rabbits does not respond histologically to small doses of thyrotropic hormone as well as the normal gland (Aron and Dobrzaniecki, 1930a, b; Eitel, Krebs and Loesser, 1933; Pieper, 1934). On the contrary, it has been reported that section of the cervical sympathetics in mice and rabbits has no effect on either the histological or metabolic effects of thyrotropic hormone (Krayer, 1933).

Friedgood and Cannon (1940) observed in four cats with bilateral cervical sympathectomy, two of which had the adrenals denervated, that thyrotropic hormone produced the same rise in basal metabolic rate as in two normal controls. Though it appeared from this literature that the results of another study would be negative, it was decided important in relation to our following study to ascertain in guinea pigs the effect of cervical sympathectomy on the metabolic response to thyrotropic hormone.

Using rats, Ring (1918) found that the cervical sympathetics were not essential for the elevation in basal metabolic rate which occurs on

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exposure to cold. Uotila and his colleagues have concluded (Uotila, 1939b, c; 1940; Dempsey, 1940) that the increase in metabolism in rats exposed to cold was due to increased thyrotropic hormone secretion regulated chiefly by the thyroxin blood level and by hypothalmic impulses which pass to the pituitary through the stalk and to a lesser degree through the cervical sympathetics. In a previous article, he (Uotila 1939a) presented evidence indicating that the cervical sympathectomy temporarily modified, perhaps through lack of vasomotor control of the thyroid, the response of the thyroid to thyrotropic action. Our object was to find whether this temporary modification is physiologically significant in relation to the observations of Ring.

I. THE EFFECT OF BILATERAL CERVICAL SYMPATHECTOMY ON THE METABOLIC RESPONSE TO THYROTROPIC HORMONE

Method: Thirty mature female guinea pigs of approximately the same age were kept in roomy cages in a constant temperature ($30 \pm 2°$ C.) room for at least two months. They were fed daily with lettuce, carrots, oats, and Purina Chow. The basal oxygen consumption was determined every four days during the latter part of the period until the animals were conditioned to the procedure. Three closed circuit metabolism machines with a glass jar containing the animals were used in the experiment. Oxygen consumption was read directly from a floater which could be read in cc. with an error of less than 0.5 per cent. The three units were contained in a constant temperature cabinet at 30 to 31° C. This is the critical temperature of guinea pigs (Manley and Marine, 1915). Absolute silence and constant light intensity were maintained in the metabolism room.

Fifteen minutes after the guinea pig had been placed in the respiratory chamber, oxygen consumption during each of six ten-minute periods was recorded. The average of the three lowest readings was reduced to Standard Temperature and Pressure, changed to calories, and thence converted into calories of heat production per kilo per hour.

The superior, middle, and in most instances the stellate ganglions, along with the cervical chain were removed bilaterally in seventeen of these animals under an ether anesthetic. The remainder were subjected to a mock operation in which the sympathetic nerves and thyroid gland were carefully avoided, though the cervical tissues were dissected. Two weeks later ten healthy animals from each group were started on daily injections of thyrotropic hormone for twelve days. Each injection according to assay was sufficient to produce a fifty per cent increase in thyroid weight in five days when injected daily. Metabolic determinations were made at two, six, ten, fourteen, and eighteen days following the first injection of hormone. The so-called "metabolic principle" which produces early metabolic increases (O'Donovan and Collip (132), and Billingsley (33)), was avoided by making all determinations at least eighteen hours subsequent to the previous injection of the extract. All animals were fasted for a like period. Rectal temperatures were taken for 3 minutes each.

Results: The averaged data obtained on the basal heat production of the ten control and the ten cervical sympathectomized guinea pigs

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prior to the administration of thyrotropic hormone are shown in Table I. It is to be noted that the average for the group subjected to to sympathectomy is 0.11 calories per kilo per hour higher than that for the group subjected to the mock operation. This difference is not statistically significant and could be due to random sampling. The operations caused no change in weight.

The effect of injection of thyrotropic hormone daily for 12 days on the basal heat production of each group is best illustrated by a graph

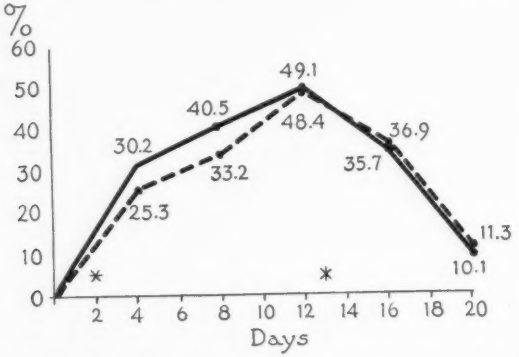

FIG. 1. Showing the average metabolic response (solid line) of the control (mock operation) and (broken line) of the sympathectomized guinea pigs to thyrotropic hormone. The stars indicate the start and end of the daily injections.

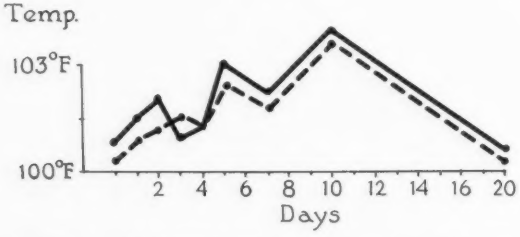

FIG. 2. Showing the rectal temperature of the two groups after the injection of thyrotropic hormone. (Broken line, sympathectomized.)

(Fig. 1). Each point in Figure 1 represents the average per cent rise in basal heat production for the group, the average per cent rise being the average of the increase of each animal above its average control.

It is to be noted that the average elevation of the sympathectomized group is slightly less than that of the mock group, though the control group during the control period had on the average a lower heat production. However, none of the differences between groups shown in Figure 1 are statistically significant, though the difference is consistent for the five consecutive determinations. If cervical sympa-

thectomy actually has a significant effect, vasomotor or otherwise, on the action of thyrotropic hormone a statistically significant difference should have been observed.

In Figure 2, the average rectal temperatures of the group approximately parallel the basal heat production. The differences are not significant.

II. THE EFFECT OF EXPOSURE TO COLD ON THE BASAL HEAT PRODUCTION OF CONTROL (MOCK OPERATION) AND BILATERAL CERVICAL SYMPATHECTOMIZED GUINEA PIGS

Methods: Guinea pigs were prepared in exactly the same manner as in the previous study. In this instance, however, the guinea pigs were paired in

TABLE I. SHOWING THE CONTROL BASAL HEAT PRODUCTION OF THE TEN "CONTROL" GUINEA PIGS SUBJECTED TO A MOCK OPERATION AND THE TEN SUBJECTED TO BILATERAL CERVICAL SYMPATHECTOMY BEFORE OPERATION AND ADMINISTRATION OF THYROTROPIC HORMONE

Controls Prior to Thyrotropic Hormone	No. of pigs	Cal./Kg./hr.		
		Max.	Min.	Ave.
Control Group—Mock Operation	10	3.01	2.58	2.82
Cervical Sympathectomy	10	3.15	2.65	2.93

TABLE II. SHOWING CONTROL BASAL HEAT PRODUCTION OF THE GUINEA PIGS TO BE SUBJECTED TO EXPOSURE TO COLD

Prior to Exposure to Cold	No. of pigs	Cal./kg./hr.		
		Max.	Min.	Ave.
Control Group—Mock Operation	11	3.41	2.30	2.87
Cervical Sympathectomy	10	3.65	2.56	2.97

regard to weight before the control determinations were made. Ten sympathectomized pigs and eleven controls were used. All gained weight after the operations. Exposure to a temperature of 7 to 14° C. for sixteen days was begun seven days after surgery. The metabolic chambers were maintained at 14 to 16° C. during the cold exposure, and the basal heat production was calculated as in the previous study.

Results: Eight of the 10 sympathectomized and 10 of the 11 control animals survived the 16-day cold exposure. The control median basal heat production of the two groups before exposure to cold is shown in Table II.

The exposure to cold caused an immediate increase in the basal heat production (figure 3) to an equal extent in both groups. The heat production then increased in the sympathectomized group and decreased in the control group until the ninth day. On the ninth day the difference of 30 per cent between the means of the two groups was

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statistically significant; the probable error was 6.27 per cent or about one-fifth the difference, which indicates that the difference is highly significant mathematically and not due to chance variation. The difference at the fifth day was questionably significant. The difference between the groups persisted until the sixteenth day of exposure, but

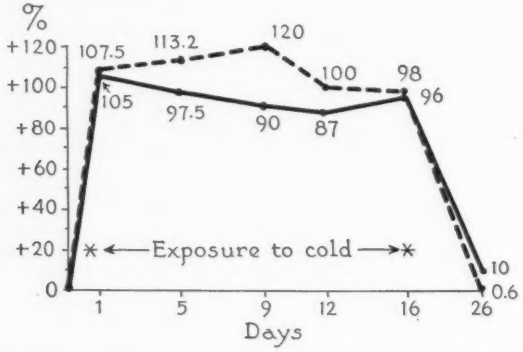

FIG. 3. Showing the mean metabolic response to cold exposure (solid line) of control (mock operation) and (broken line) of cervical sympathectomized guinea pigs.

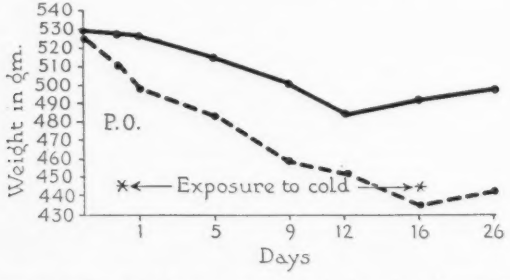

FIG. 4. Showing the weight loss in (solid line) the control and (broken line) the cervical sympathectomized guinea pigs on exposure to cold. P.O. indicates 7 days post-operative.

it was not a significant difference. Ten days after the exposure the guinea pigs had returned to the normal or pre-exposure level.

It is to be noted in Figure 4 that both groups lost weight during the cold period. At the end of the exposure, the sympathectomized group averaged 56 grams less in body weight. Ten days after restoration to room temperature this difference was still present. It is to be noted also that the basal heat production per kilogram body weight was approximately the same in the two groups at the end of the exposure and 10 days later regardless of the divergence in body weight and to a less extent presumably in surface area. Hence, neither of these two factors are logically concerned in the differences in heat production.

It was observed that the sympathectomized animals during exposure were irritable and tended to shiver when moved from the cage in the cold room to the respiratory chamber; this was rarely noted in the controls.

The Effect of Cervical Sympathectomy in the Serum Cholesterol Level in Guinea Pigs. In the course of this work, the serum cholesterol was determined in five pairs of young female guinea pigs, in five bilateral cervical sympathectomy had been performed up to six months previously, the superior cervical ganglion having been removed presumably to prevent regeneration. No difference in the blood cholesterol values was found, the average for the mock-operated controls being 76.28 mg. per cent and of the sympathectomized being 76.16 mg. per cent.

DISCUSSION

Our results show that bilateral sympathectomy does not modify the metabolic response of the thyroid to thyrotropic hormone in guinea pigs. This confirms the observations of Krayer (1933) in mice and rabbits, and Friedgood and Cannon (1940) in cats. One should not expect it to do so unless the operation significantly decreases the blood flow to the thyroid gland.

Our results strongly indicate that removal of the cervical sympathetics causes an increased metabolic response to cold in guinea pigs during the first 9 to 12 days of the exposure. This is not contrary to the observations of Ring (1918) in rats. He removed bilaterally the superior cervical sympathetic gamglion in five rats and studied the metabolic response to cold before exposure (average of "at least two determinations"), within 24 hours and one week after exposure. He observed no difference between these five rats and five controls. We also noted no differences when the basal heat production was studied under the same conditions as in our guinea pigs; our differences were noted only during the first 9 to 12 days of exposure. Uotila (1939a) studied only the cell heights of the thyroid in rats. He found that after 10 to 12 days the increase in cell height decreased in operated controls and decidedly increased over the controls in the bilaterally cervical sympathectomized rats. It appeared as though in the absence of sympathetic control thyrotropic hormone was excessively produced. We believe our results check well with Uotila's observations, except for the exact time intervals and his initial changes which may not be significant. Thus, we interpret our results as indicating that the cervical sympathetic serves to regulate in an inhibitory direction the output of thyrotropic hormone or thyroid secretion on exposure to cold. Since the cervical sympathetic is so rich in vasoconstrictor nerves, it is most plausible to assume for the present that the inhibitory regulation is secondary to the activity of the vasoconstrictor nerves.

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SUMMARY AND CONCLUSIONS

Following control determinations of the basal heat production, ten bilateral cervical sympathectomized and ten control (mock operation) guinea pigs were injected daily for 12 days with thyrotropic hormone. No significant difference was observed in the heat production and rectal temperature of the two groups of animals. The cervical sympathetic nerves do not affect the metabolic response to thyrotropic hormone in the guinea pig.

After determining their basal heat production, ten bilateral cervical sympathectomized and eleven control (mock operation) guinea pigs were exposed in a cold room (7° to 14° C.) for 16 days. During this period the basal heat production was determined in a cold respiratory chamber (14° to 16° C.). It was found that heat production was greater after the first day in cervical sympathectomized animals than in controls. The difference was decidedly significant on the 9th day but not on the 12th and 16th days. These results are interpreted as indicating that the cervical sympathetic nerves inhibit, perhaps by vasoconstrictor activity, either the production of thyrotropic hormone or the output of thyroid secretion.

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THE COLLECTION AND LOSS OF RADIOACTIVE IODINE COMPARED WITH THE ANATOMIC CHANGES INDUCED IN THE THYROID OF THE CHICK BY THE INJECTION OF THYROTROPIC HORMONE[1]

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THE MODE of action of thyrotropic hormone has been the subject of intensive study. This is not surprising in view of the fact that of the various active principles of the anterior hypophysis the thyrotropic fraction is one whose anatomic and physiologic effects are most readily adaptable to quantitative observations. Furthermore much interest in the effects of thyrotropic hormone arises from the fact that its injection is a practical means of producing experimentally in the laboratory animal a goiter which is both hyperplastic and hyperfunctioning (Lambie, 1939).

Marine (1915) was the first to study the marked capacity of the thyroid gland to collect exogenous iodine. Both in vivo experiments (Marine, 1915) and in vitro perfusion studies (Marine and Feiss, 1915) disclosed that the capacity to collect iodine increased with the degree of hyperplasia of the gland. Marine and Rogoff (1916a and b) demonstrated that the rate of collection of an injected dose of iodine was greatest during the first ten minutes.

In recent years this observation has been repeatedly confirmed by studies employing radioactive iodine (Hertz, Roberts and Evans, 1938; Hamilton and Soley, 1940; Lein, 1941; Leblond and Süe, 1941). Hertz and his associates (1939) also found that the collection of radioactive iodine by the thyroid of the rabbit paralleled the in-

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creases in basal metabolic rate, the weight of the thyroid gland and the mean acinar cell height which resulted from the injection of thyrotropic hormone, but that the collection did not parallel the increases beyond a certain ceiling. Leblond and Süe (1940) found that thyrotropic hormone augmented the capacity of the thyroid to fix radioactive iodine.

The present study was undertaken in an effort to clarify further the relationships between the anatomic effects of the injection of thyrotropic hormone and the accompanying changes in thyroid function, as indicated by the rate at which the thyroid collected and lost radioactive iodine. The chick was selected as the experimental animal because Rawson and Salter (1940) had observed that the uniform histologic structure of chick thyroid and the sensitivity to thyrotropic hormone made it particularly suited for the studies of the physiologic effects of this hormone.

METHODS

Red Rock sex-linked hybrid cockerels were employed as experimental animals. They were fed a standard starter mash estimated to contain 400 to 600 micrograms of sodium iodide per kilogram, starting when forty-eight hours old, and were housed in large, constant temperature brooders which permitted segregation of various experimental groups from one another.

An artificially radioactive isotope of iodine having an atomic weight of 131 and a half life of eight days was used. This was prepared by fusing a mixture of cobalt and copper tellurides onto a knurled copper target surface, which was then bombarded with deuterons in the cyclotron. After bombardment the surface of the target was removed with a small end mill. The coarse powder resulting was placed in an all-glass distilling apparatus and 0.5 mg. of iodine added as a standard potassium iodide solution. Thirty cubic centimeters of 6M nitric acid were added, whereupon the iodine was distilled from the solution and caught in 15 cc. of carbon tetrachloride. The distillate was transferred to a 125 cc. Squibb separatory funnel and washed twice with distilled water to remove the nitric acid. The carbon tetrachloride phase was then shaken with 20 cc. of distilled water containing sufficient sodium bisulfite to reduce all the iodine to iodide. The sodium iodide resulting collected in the aqueous phase, which was then separated from the carbon tetrachloride phase. The carbon tetrachloride was washed once with 10 cc. of distilled water and the wash water added to the original aqueous extract, which was then filtered to remove any droplets of carbon tetrachloride remaining. Recovery of iodine by this procedure usually averaged about 80 per cent. The specific activity of I^{131} so obtained varied from 1 to 10 microcuries per microgram.

The radioactive iodine solution was thereupon diluted with distilled water and sodium iodide, as required, to a strength of 8 micrograms of sodium iodide per cubic centimeter, with a specific activity at the start of the experiment not exceeding 2 microcuries per microgram. Twenty-five hundredths cubic centimeter of this solution was injected subcutaneously into the thigh of each chick by means of a 0.25 cc. tuberculin syringe. The puncture hole was carefully sealed with collodion to prevent leakage.

All animals were killed by a blow on the head. Animals which were given radioactive iodine were killed four hours after its injection. All thyroids were removed within ten minutes and special care was exercised in removing the thyroids of animals given radioactive iodine to avoid loss or contamination. Each pair of glands was placed in an individual digestion bottle containing 5.0 cc. of a solution of 1 per cent sodium hydroxide and 1 per cent sodium iodide. Dissecting instruments were rinsed in a solution containing silver nitrate, rerinsed in cold water and dried with clean towels after each chick had been dissected.

The thyroids were allowed to remain in the digestion mixture approximately a week, at the end of which time all thyroid tissue was considered to be completely dissolved. Two-tenths cubic centimeter of the resulting solution and 0.1 cc. of a solution of silver nitrate containing 1 mg. of silver ion per cubic centimeter were pipetted onto silver planchettes 1 inch (2.5 cm) in diameter and 30 mils (0.076 cm.) thick. This was allowed to dry and was then measured on a thin-window beta-ray counter. The count so obtained was compared with a standard blank prepared at the time when the solution was injected into the chicks by pipetting 0.25 cc. of the original radioactive iodine solution into a bottle containing 4.75 cc. of the digestion mixture.

It was not considered advisable to weigh the thyroids of chicks which were to be used for determination of radioactive iodine because of the risk of loss or contamination of radioactivity entailed by this procedure. Tissue for determination of thyroid weight and histologic sections was obtained from other chicks similarly treated. The thyroids of these chicks were carefully removed and cleaned, and were weighed while wet in a Roller-Smith torsion balance. Each pair of thyroids was then placed in an individual bottle containing 5 per cent solution of formalin in which it was allowed to remain for twenty-four hours. After this, paraffin sections 10 microns thick were prepared in the usual manner and stained with hematoxylin and eosin. Mean acinar cell heights were measured by careful adherence to the technic of Rawson and Salter (1940). The heights of 100 cells, each a representative of a separate acinus, from the thyroid of each chick were measured.

RESULTS

Experiment 1. The effect of varying doses of thyrotropic hormone.— One hundred twenty[2] chicks were divided into five groups. The first group received 1 cc. of distilled water subcutaneously each day for four days and served as controls. The other groups were given daily injections for four days of $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$ or 1 Junkmann-Schoeller unit of thyrotropic hormone,[3] respectively, in 1 cc. of water. Twenty-four hours after the last injection, radioactive iodine was given to all groups, which were then killed four hours later.

The data obtained are condensed into table 1 and graphically portrayed in figure 1. With increasing doses of thyrotropic hormone both

[2] The numbers given in the text are the numbers of chicks at the beginning of the experiments. The numbers given in the tables are the numbers of chicks that survived to the end of the experiment and on which the data of the tables are based.

[3] The thyrotropic hormone used in this study was antuitrin T, generously supplied by Dr. E. A. Sharp of Parke, Davis & Company.

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TABLE 1. THE EFFECT OF VARYING DOSES OF THYROTROPIC HORMONE ON COLLECTION OF RADIOACTIVE IODINE, MEAN ACINAR CELL HEIGHT AND WEIGHT OF THYROID GLAND

Thyrotropic hormone, units per day	Animals	Mean cell height, microns	Animals	Thyroid weight, mg.	Animals	Collection of radioactive iodine, per cent of injected dose
0 (Controls)	5	1.9±0.1[1]	5	3.3±0.4[1]	9	8.2±3.4[1]
1/8	4	4.4±0.1	4	8.3±1.1	9	35.3±2.4
1/4	4	5.8±0.1	4	11.4±1.4	9	43.5±2.9
1/2	5	6.9±0.1	5	12.8±0.8	7	43.8±2.6
1	5	7.9±0.2	5	17.7±4.2	9	40.8±3.0

[1] The mean of each group is given with the standard error of the mean.

the weights of the thyroid glands and the mean acinar cell heights continued to increase, although both categories showed some tendency to level off with the larger doses. The capacity of the thyroid to collect radioactive iodine, however, did not increase with doses larger than $\frac{1}{4}$ unit of the hormone per day.

Experiment 2. The effect of repeated doses of thyrotropic hormone.—A total of 278 chicks were employed in this experiment. Observations were repeated in whole or in part on six different occasions. As the results of the separate experiments agreed very closely, all of the data were combined and condensed into table 2. In each experiment the chicks were divided into two groups. The first group, which served as

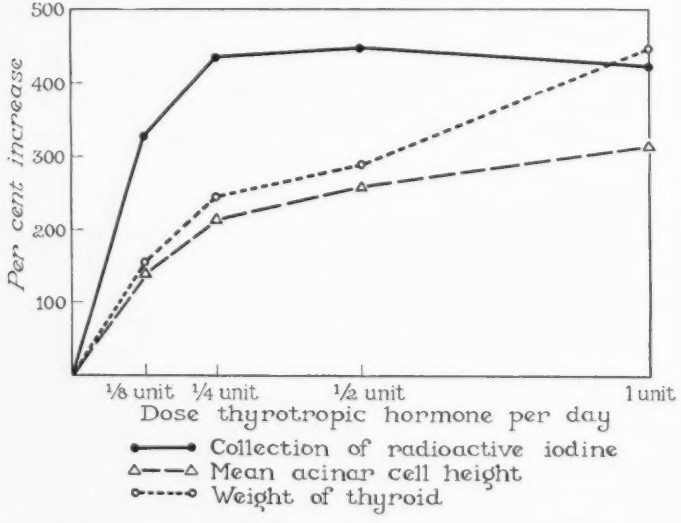

FIG. 1. The effect of varying doses of thyrotropic hormone. The hormone was injected daily for four days and the animals were killed twenty-four hours after the fourth injection.

controls, received 1 cc. of distilled water subcutaneously daily; the second group, the test group, received 1 unit of thyrotropic hormone daily in 1 cc. of distilled water subcutaneously. Beginning twenty-four hours after the first injection, certain animals from each group were killed twenty-four hours after each succeeding injection for five days. Control animals were killed at the beginning of the experiment and at each succeeding twenty-four hour period for five days.

The results are shown in figure 2. The mean acinar cell height increased rapidly after the initial dose, and then increased by succes-

TABLE 2. THE EFFECT OF REPEATED DOSES OF THYROTROPIC HORMONE ON COLLECTION OF RADIOACTIVE IODINE, MEAN ACINAR CELL HEIGHT AND WEIGHT OF THYROID GLAND

Time after initial dose, hrs.	Animals	Mean cell height, microns	Animals	Thyroid weight, mg.	Animals	Collection of radioactive iodine, per cent of injected dose
0 (Controls)	8	1.8±0.1[1]	5	3.0±0.3[1]	12	6.0±1.8[1]
24	9	4.9±0.1	5	5.0±0.3	27	8.2±0.9
48	8	5.9±0.1	5	8.1±0.4	26	20.8±2.6
72	8	7.4±0.2	5	17.5±1.6	18	42.0±3.0
96	10	8.4±0.1	5	19.9±3.2	33	49.5±2.2
120	8	9.3±0.2	5	33.4±2.7	17	50.9±4.6
Additional controls						
24					16	7.7±1.4
48					18	9.2±1.0
72					8	7.4±3.4
96					27	8.1±1.3
120	6	1.9±0.1	5	3.3±0.4	19	10.3±1.4

[1] The mean of each group is given with the standard error of the mean.

sively smaller increments after each subsequent dose. The weight of the thyroid, on the other hand, increased slowly at first, lagging behind the mean acinar cell height curve; but after the third day the mean thyroid weight increased with ever larger increments, overtaking and far exceeding the increase in the mean acinar cell height by the end of the fifth day. The capacity of the thyroid to collect radioactive iodine showed no change whatsoever in the first twenty-four hours, despite the large increase in cell height and in thyroid weights which had occurred. From twenty-four to seventy-two hours after the beginning of the experiment, however, radioactive iodine was collected in increasing amounts. Thereafter, collection subsequently leveled off, although rapid increase in thyroid weight continued to take place.

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Experiment 3. The effect of a single injection of thyrotropic hormone. —A total of 144 chicks used in this experiment were divided into two groups. The first group, which served as a control group, received a single injection of 1 cc. of distilled water, while the chicks in the second group received 1 cc. of water containing 4 units of thyrotropic

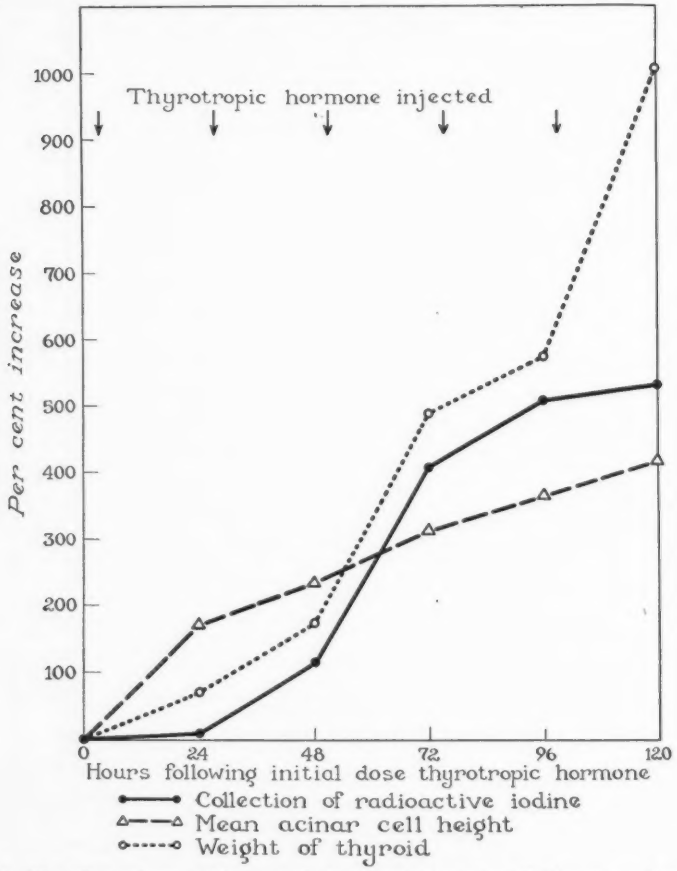

FIG. 2. The effect of repeated doses of thyrotropic hormone. Each group of animals was killed 24 hours after the last injection.

hormone. Control chicks were killed at the start of the experiment and test chicks from each group were killed each twenty-four hours thereafter for the ensuing four days. A summary of these data is shown in table 3.

The results of this experiment are shown in figure 3. As in the preceding experiment a large initial increase in the mean cell height had occurred in the first twenty-four hours. The mean height of the acinar cells increased but slightly during the second day, and thereafter de-

TABLE 3. THE EFFECT OF A SINGLE INJECTION OF THYROTROPIC HORMONE ON COLLECTION OF RADIOACTIVE IODINE, MEAN ACINAR CELL HEIGHT AND WEIGHT OF THYROID GLAND

Time after initial dose, hrs.	Animals	Mean cell height, microns	Animals	Thyroid weight, mg.	Animals	Collection of radioactive iodine, per cent of injected dose
0 (Controls)	13	1.8±0.1[1]	5	3.0±0.4[1]	12	6.0±1.8[1]
24	8	4.7±0.1	4	6.5±0.2	10	7.1±0.6
48	9	5.2±0.4	4	8.2±1.0	9	14.2±2.3
72	10	4.5±0.2	4	4.8±0.7	9	10.5±1.0
96	12	4.1±0.2	5	3.4±0.6	8	22.7±2.6

Additional controls

Time after beginning of experiment, hrs.	Animals	Mean cell height, microns	Animals	Thyroid weight, mg.	Animals	Collection of radioactive iodine, per cent of injected dose
24					10	8.8±1.6
48					7	5.0±1.3
72					7	5.6±2.0
96	11	2.0±0.1	5	3.3±0.2	9	7.4±1.8

[1] The mean of each group is given with the standard error of the mean.

creased cell heights were recorded. The thyroids increased in weight by equal increments during the first two days but lost weight during the third and the fourth days, returning to the original weight of the controls. No significant increase in the collection of radioactive iodine occurred until the second day and this function of the gland did not reach its maximum until the fourth day, when as we have shown the anatomic changes were regressing. Unfortunately, the experiment was not continued further as this result was not foreseen.

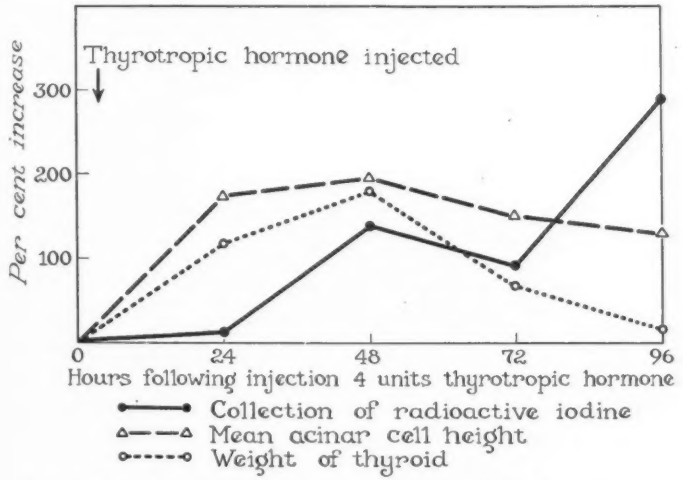

FIG. 3. The effect of a single injection of 4 units of thyrotropic hormone.

TABLE 4. THE EFFECT OF THYROTROPIC HORMONE ON THE RATE OF LOSS OF RADIOACTIVE IODINE PREVIOUSLY COLLECTED BY THE THYROID

Time after initial dose, hrs.	Animals	Percentage of injected dose present	Percentage of loss following thyrotropic hormone
0 (Controls)	10	17.20 ± 4.00[1]	0
24	10	4.00 ± 1.80	77
48	10	1.20 ± 0.25	93
72	10	0.70 ± 0.22	96
72 (Controls)	10	8.80 ± 2.60	49

[1] The mean of each group is given with the standard error of the mean.

Experiment 4. The effects of thyrotropic hormone on the loss of radioactive iodine from the thyroid.—A total of fifty chicks were given 0.5 micrograms of sodium iodide containing radioactivity when they were forty-eight hours old, before they had been fed or watered. Food and water were then provided for the ensuing twenty-four hours, when the experiment was begun. Ten control chicks were killed at the start of the experiment and ten additional control chicks were killed seventy-two hours later. The remaining chicks (thirty) were given 1 unit of thyrotropic hormone by injection each twenty-four hours for three

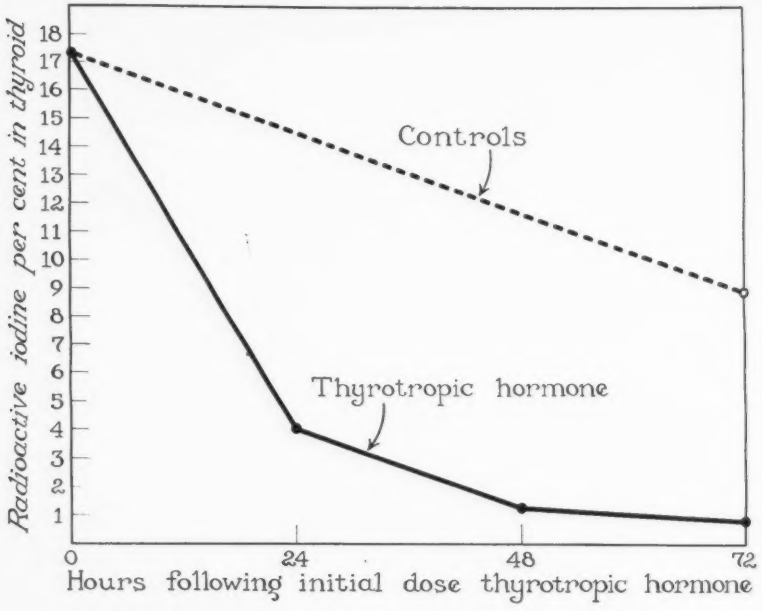

FIG. 4. The effect of thyrotropic hormone on the loss of iodine previously stored in the thyroid.

days. A group of ten test animals was then killed twenty-four hours after each succeeding injection.

The results are given in table 4 and shown graphically in figure 4. The loss of the iodine which had been stored in the gland was greatly accelerated by each successive injection of thyrotropic hormone. Seventy-seven per cent of the quantity initially present in the gland was lost during the first twenty-four hours, 70 per cent of that which remained was lost during the second twenty-four hours and 42 per cent of that which still remained was lost during the third twenty-

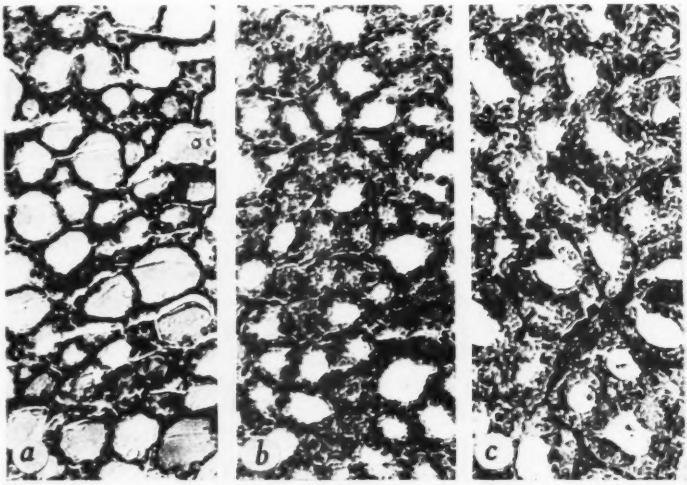

FIG. 5. Histologic appearance of thyroids stimulated with thyrotropic hormone (magnification ×300). On the left is a normal control (*a*); in the center (*b*) a thyroid removed twenty-four hours after a single dose of thyrotropic hormone; on the right (*c*) a thyroid twenty-four hours after the fifth of five daily injections of 1 unit of thyrotropic hormone.

four hours. Thus in seventy-two hours, 96 per cent of the collected radioactive iodine had disappeared from the chicks which were given thyrotropic hormone, compared with 49 per cent lost by the controls.

COMMENT

Changes in the weight or the size of the thyroid gland may be due to alterations in (1) vascularity; (2) stroma; (3) colloid; (4) size of the acinar cells or (5) the number of acinar cells. A comparison of the histologic appearance of the thyroid stimulated by an injection of thyrotropic hormone with that of a normal gland suggests that several of these factors may have been altered (fig. 5). There is an obvious increase in the size of the acinar cells and an increase in the blood supply to the gland but a decrease in the quantity of colloid material. Accurate measurements of the mean acinar cell heights provide informa-

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tion about one of these factors, cell size or hypertrophy, but changes in the weights of the glands provide no more than conjecture about the other factors suggested.

A comparison of the curves depicting changes in the mean acinar cell heights with those describing the weights of the thyroids, after injection of thyrotropic hormone (figs. 2 and 3), suggests that the initial effect of the hormone was immediate, inducing marked hypertrophy of the cellular epithelium accompanied by a loss of colloid, for the gland weights did not increase proportionately to increases in cell heights. But after continued hormonal stimulation for several days, disproportionate increases in thyroid weights occurred, which, in view of the lack of colloid materials, may be interpreted as a true cellular hyperplasia.[4]

We had expected to find that the thyrotropic hormone would produce an early and pronounced increase in the quantity of injected radioactive iodine collected by the thyroid but many experiments have failed to disclose any change in this function until after histologic changes have become marked. There was an initial lag of forty-eight hours before any increase in ability of the thyroid to fix iodine was apparent. With continued stimulation (fig. 2) the increase in the capacity of the thyroid to collect radioactive iodine did not keep pace with the increasing weight of the gland. When varying degrees of stimulation were produced, we learned that after injection of small amounts of thyrotropic hormone, increases in the collection of radioactive iodine exceeded anatomic changes. However, when larger quantities of the hormone were given, the collection of iodine did not increase further in proportion. It would seem that the rapidly growing and proliferating thyroid has for a time at least a diminished capacity for collecting radioactive iodine. It is of interest to recall the work of Gorbman and Evans (1943), who demonstrated that fetal rat thyroids were incapable of collecting iodine until the nineteenth day, and even then the capacity for collecting iodine was much less than that observed on the twenty-first day.

Because the thyroid gland secretes iodine, as the thyroid hormone, as well as collects it from the blood stream, we undertook a study of this secreting function. We had found in previous studies that the rate of loss of radioactive iodine from the thyroid of normal chicks was relatively small and was more or less constant; approximately 15 per cent of the iodine present in the thyroid was lost each day for the first few days. The average daily loss from thyroids of control chicks (experiment 4) was of a similar magnitude; for example, 49 per cent in three days.

The injection of thyrotropic hormone, however, produced an early and striking acceleration in the rate with which stored radioactive iodine was lost from the thyroid. Seventy-seven per cent of the quan-

[4] By hypertrophy is meant increase in size of individual cells as measured by mean acinar cell height; by hyperplasia is meant the formation of new cells.

tity initially present was lost the first day, 70 per cent of the quantity remaining was lost the second day and 42 per cent of the quantity which still remained disappeared by the third day. This latter figure may be inaccurate because of the minute quantities of iodine which were involved.

This observation takes on added significance if one assumes that the radioactive iodine initially present had been transformed in the glands into hormone iodine, an assumption which the studies of Lein (1943) and of others strongly suggest. If this is the case, then the 17.2 per cent of the injected iodine, present at the beginning of the experiment, may be considered as labeled thyroid hormone in the gland and the percentage of iodine loss after each successive injection of thyrotropic hormone (77 per cent, 70 per cent, 42 per cent) may be considered to represent the proportion of all of the stored thyroid hormone which was secreted as the result of each injection of thyrotropic hormone.

We observed a striking difference between the effect of thyrotropic hormone on the collection of iodine by the thyroid and the effect on its subsequent loss from the gland. The latter occurred at least as promptly as did anatomic changes; the former was delayed at least twenty-four hours longer.

How does the thyroid stimulated with thyrotropic hormone become capable of collecting an increased quantity of iodine? A review of the data presented suggests several possible explanations but does not provide conclusive evidence for them. Since thyrotropic hormone evidently produces such a prompt and marked loss of stored iodine from the gland, the increased capacity to collect iodine which appears somewhat later may possibly be in part a reflection of the resulting deficiency of iodine stores in the gland. The data suggest that stores of iodine are depleted some time before increased collection of iodine can take place.

It is likely, however, that other factors are also involved. Cell growth, as measured by increasing mean acinar cell height, has been present at least twenty-four hours before an initial increase in capacity to collect iodine is observed. It is also possible therefore that the increased capacity to collect iodine, when it does occur, may be in part dependent on the increased quantity of cellular tissue present as the result of such growth. This might be construed to indicate that thyrotropic hormone does not affect collection of iodine directly, but only indirectly, by augmenting the quantity of thyroid tissue present.

Despite the fact that it is slow to appear, the increase in collection of iodine which follows injection of thyrotropic hormone might still be considered in part a direct effect of the hormone if it could be shown that injection of the hormone ultimately produced a proportionately greater increase in capacity to collect iodine than in thyroid cell mass. In the experiments which have been presented, proportionally greater collection occurred only in the glands stimulated with submaximal

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doses of thyrotropic hormone (fig. 1) and those measured ninety-six hours after a single injection of a maximal dose (fig. 3). Further accurate observations under varying conditions will be required to establish the mechanism involved.

SUMMARY

After the injection of thyrotropic hormone into the chick, thyroid hypertrophy, as measured by an increase in the mean acinar cell height, occurred rapidly and was conspicuous within twenty-four hours. No increase in the collection of radioactive iodine, however, occurred until forty-eight hours had elapsed.

When stimulation of the thyroid with thyrotropic hormone had continued to a point where hyperplasia (as measured by a marked increase in thyroid weight) was increasing rapidly, the collection of radioactive iodine by the thyroid did not increase proportionally.

With mild degrees of thyrotropic hormone stimulation, the increase in the collection of radioactive iodine exceeded the anatomic changes but with more marked degrees of stimulation further increase in collection of radioactive iodine did not occur.

There is reason to believe that rapidly proliferating thyroid tissue may have a diminished capacity to collect radioactive iodine from the blood stream.

Thyrotropic hormone produced a prompt and early acceleration in the rate with which iodine previously stored in the thyroid was lost from it. It would seem that this is a measure of the accelerated secretion of thyroid hormone from the gland induced by the thyrotropic stimulation.

The authors wish to acknowledge their indebtedness to the members of the staff of the Radioactivity Center of the Department of Physics, Massachusetts Institute of Technology, for their active participation in assembling the data; to Dr. G. M. Higgins for many suggestions and for the use of his laboratory at the Institute of Experimental Medicine of the Mayo Foundation and to Dr. J. H. Means and Dr. S. F. Haines for their stimulating advice and encouragement.

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A COMPARISON OF THE EFFECT OF THIOURACIL AND OF INJECTED THYROTROPIC HORMONE ON THE COLLECTION OF RADIOACTIVE IODINE AND THE ANATOMIC CHANGES INDUCED IN THE THYROID OF THE CHICK[1,2]

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MACKENZIE AND MACKENZIE (1943) and Astwood and his associates (1943) found that certain drugs of the aniline and thiourea series produce marked hyperplasia of the thyroid of the rat associated with evidences of diminished thyroid function. The goitrogenic action of these drugs was found to depend on the integrity of the anterior lobe of the pituitary gland and the evidence indicated that it was a result of increased secretion of thyrotropic hormone. In contrast to other active goitrogens, these substances produced thyroid hyperplasia even in the presence of large quantities of iodine. Astwood and others concluded that they acted by blocking the synthesis of thyroid hormone.

Using radioactive iodine, Franklin and Chaikoff (1943) and Schachner and his associates (1943) found that sulfanilamide in "in vitro" studies inhibited the formation of thyroxine and diiodotyrosine by thyroid slices but failed to limit the capacity of such slices to collect radioactive iodine. Later, Franklin and his associates (1944) in a small series of animals found that thiouracil limited the capacity of the thyroid of the rat to collect radioactive iodine. Rawson, Tannheimer and Peacock (1944) found that the thyroid glands of rats given

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[2] Abridgment of part of thesis submitted by Dr. Larson to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of M.S. in Medicine.

thiouracil collected but 10 per cent of an injected dose of radioactive iodine, whereas the thyroids of control animals collected 56 per cent. Baumann and his associates (1944) reported that rabbits treated with thiourea and thiouracil lost iodine from their thyroids and Astwood and Bissell (1944) showed that the iodine content of the thyroid of the rat declined in direct proportion to the length of time that thiouracil was given.

Mixner and his associates (1944) studied the effect of thiourea and of thiouracil on the thyroid of the chick and found these substances to be goitrogenic for this animal as for the rat. They found that the response differed in degree among various breeds of chickens and they obtained a consistently larger response among pullets than among cockerels.

The present study was undertaken in an attempt to study somewhat further the mode of action of thiouracil on the thyroid. In a previous study (Keating, Rawson, Peacock and Evans, 1945) it was shown that histologic changes in the thyroid of the chick, induced by thyrotropic hormone were correlated, within certain limits, with its capacity to collect radioactive iodine. Hyperplastic thyroids were found to have, at least for a time, a relatively diminished capacity to collect radioactive iodine. However, because of the increase in the size of the thyroid resulting from stimulation by thyrotropic hormone, the proportion of injected iodine collected by such animals was greater than that collected by controls.

The hyperplastic, hypofunctioning thyroid which develops on the administration of thiouracil to experimental animals has been assumed to be due to an increased secretion of thyrotropic hormone by the pituitary gland. It has been previously recognized, of course, that the injection of thyrotropic hormone results in the development of a hyperplastic, hyperfunctioning goiter. A comparison of the histologic changes in the thyroids with their capacities to collect radioactive iodine when stimulated either by thiouracil or by thyrotropic hormone offered direct means, it seemed to us, of investigating the physiologic differences between these two goitrogenic agents.

METHODS

White Leghorn cockerels were used. We selected the chick for this study because it had previously been found an ideal test subject for the study of the histologic and physiologic effects of thyrotropic hormone (Rawson and Salter, 1940). Cockerels were selected because their thyroids were found to be more uniform than those of pullets (Rawson and his associates, 1944). An artificially radioactive isotope of iodine having an atomic weight of 131 and a half life of eight days was used. The technics employed have been described in a previous publication.

RESULTS

1. Anatomic effects of thiouracil.—A total of 100[3] chicks forty-eight hours old were divided into six groups. The first group of fifteen chicks served as a control group. They were killed in groups of five, at the start of the experiment and at ten and fifteen days thereafter. A second group of twenty-five chicks were given 0.1 per cent of thiouracil in their drinking water and were likewise killed in groups of five, after

TABLE 1. EFFECT OF THIOURACIL AND THYROTROPIC HORMONE ON THE MEAN ACINAR CELL HEIGHT AND ON THE WEIGHT OF THE THYROID GLANDS OF CHICKS

	Treatment, days	Animals	Mean acinar cell height in microns	Weight of thyroid gland, mg. per 100 gm. of body weight	Absolute weight of thyroid gland, mg.
Controls	0	5	1.5±0.04[1]	5.4±0.4[1]	2.5±0.3[1]
Thiouracil administered	3	5	1.9±0.4	5.7±0.6	2.8±0.3
	5	5	3.1±0.2	9.4±1.4	5.7±0.7
	7	5	6.5±0.3	9.2±1.7	6.9±1.3
	10	4	8.9±0.5	14.0±1.8	11.3±1.2
	15	5	10.4±0.2	29.8±6.3	20.9±2.8
	10[2]	5	5.2±0.2	12.7±0.7	12.2±1.2
Thyrotropic hormone, ¼ unit daily	1	5	4.7±0.3	5.4±1.2	2.9±1.3
	3	5	6.2±0.4	10.6±1.0	5.8±0.5
	7	5	8.3±0.2	21.4±2.1	16.3±1.5
	10	5	7.9±0.3	18.2±2.5	14.1±2.5
	10[2]	5	5.8±0.3	14.7±1.5	16.0±1.5
Thyrotropic hormone, 1 unit daily	1	5	4.2±0.3	6.4±0.9	3.5±0.4
	3	5	8.0±0.2	15.4±1.7	8.2±1.0
	7	5	9.4±0.2	28.1±4.5	20.2±3.4
	10	4	10.1±0.1	41.0±3.2	30.7±3.0
	10[2]	4	5.5±0.1	20.8±2.9	20.2±2.4
Additional controls	10	5	4.4±0.6	6.3±1.1	5.7±1.1
	15	4	4.0±0.3	6.0±0.9	6.3±1.1

[1] Indicate standard error of mean.
[2] Animals killed five days after withdrawal of medication.

three, five, seven, ten and fifteen days of administration of thiouracil. For comparison with chicks of group 2 a third group of twenty chicks were given ¼ unit of thyrotropic hormone[4] in 1 c.c. of distilled water each day and were killed in groups of five at one, three, seven, and ten days of treatment. A fourth group of twenty chicks were given 1 unit of thyrotropic hormone daily and were killed after one, three, seven and ten days of treatment.

[3] The numbers given in the text are the numbers of chicks at the beginning of the experiments. The numbers given in the tables are the numbers of chicks that survived to the end of the experiments and on which the analyses are based.

[4] Thyrotropic hormone used was "antuitrin T" generously supplied by Dr. E. A. Sharp, Parke, Davis & Company. "Units" refer to Junkeman-Schoeller units.

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In addition, ten chicks were given 0.1 per cent of thiouracil in their drinking water for ten days, after which the drug was withdrawn. Five of these ten animals were killed at two days and five at five days after removing the drug from the chicks' drinking water. Other groups of five chicks each were given $\frac{1}{4}$ unit or 1 unit of thyrotropic hormone subcutaneously each day for ten days. Injections were then discontinued and the animals were killed five days later.

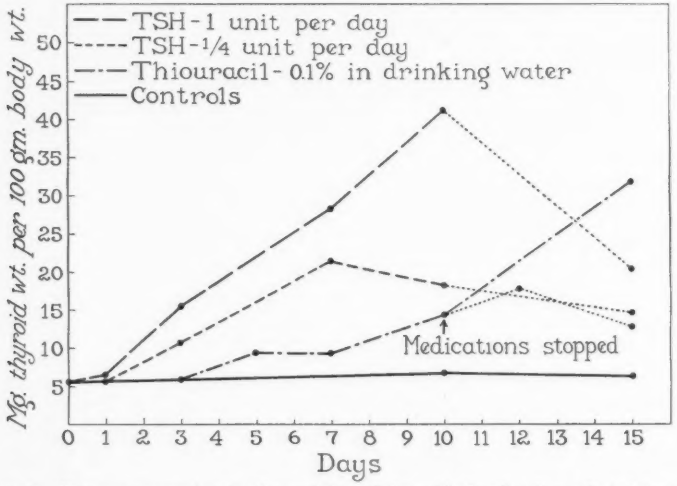

FIG. 1. Changes occurring in the weights of thyroid glands after administration of thiouracil or injection of thyrotropic hormone (TSH). The dotted portions of each curve between the tenth and fifteenth day indicate groups from which medication was withdrawn on the tenth day.

The data obtained in this experiment were summarized and condensed (table 1). Graphs of the changes which occurred in the weights of the thyroids of these chicks during the test period are shown (fig. 1). The weights of the thyroids of chicks which received thyrotropic hormone showed marked increases by the end of the third day, although little or no changes were recorded in the first twenty-four hours. In contrast to these data no increases of thyroid weights were recorded for chicks which received thiouracil until the fifth day and even then the changes were minimal. The thyroids of chicks into which 1 unit of thyrotropic hormone was injected daily continued to increase in weight until administration of the hormone was discontinued on the tenth day. After administration of thyrotropic hormone had been discontinued for five days the weight of the glands had decreased nearly to normal levels (fig. 1). The thyroids of chicks which had received $\frac{1}{4}$ unit of thyrotropic hormone daily, however, failed to increase in weight after the seventh day and showed much less regression five days after the injections were stopped on the tenth day.

The thyroids of chicks taking thiouracil daily increased for two days after its withdrawal and five days after administration of the drug was stopped they still were as large as on the last day of treatment.

The changes which occurred in the mean acinar cell heights of these various chick thyroids have been contrasted graphically (fig. 2). The mean acinar cell heights of thyroids of animals treated with thyro-

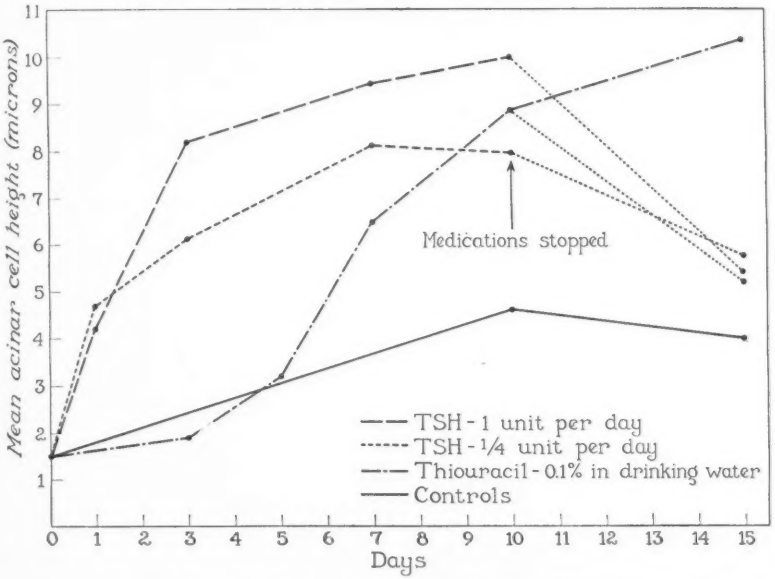

FIG. 2. Changes occurring in mean acinar cell height of thyroid glands after administration of thiouracil compared with those following injection of thyrotropic hormone (TSH). The dotted portions of each curve between the tenth and the fifteenth day indicate groups from which medication was withdrawn on the tenth day.

tropic hormone increased precipitously during the first twenty-four hours and then gradually leveled off by the tenth day, when all injections of thyrotropic hormone were discontinued. Five days later the mean acinar cell heights of the thyroids of these animals, previously treated with thyrotropic hormone, had markedly decreased but were still above control levels. No significant increase over control levels occurred in the mean acinar cell height of thyroids stimulated with thiouracil for the first five days. By the seventh day marked stimulation had occurred and it is of interest to note that the increases in the mean acinar cell heights, which were not apparent until after the fifth day of treatment with thiouracil, thereafter described a curve readily comparable with those depicting the changes in thyroids of the groups given thyrotropic hormone (fig. 2). After fifteen days of

treatment the thyroids of animals given thiouracil showed mean acinar cell heights which were identical with those obtained by the daily injection of 1 unit of thyrotropic hormone for ten days (fig. 2). The withdrawal of thiouracil from the drinking water likewise led to a decrease in the mean acinar cell heights to an extent equal to those recorded for thyroids of chicks of the other two groups.

A comparison of the weights of the thyroids with their mean acinar cell heights among chicks treated with thiouracil is shown in figure 3.

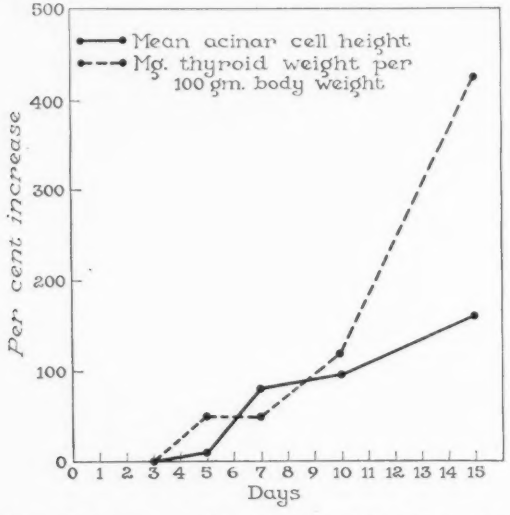

FIG. 3. Comparison of changes occurring in weight of thyroid gland and mean acinar cell height with continued administration of thiouracil.

The values are expressed as percentage increases over the control levels. Except for an initial increase of thyroid weights recorded on the fifth day, both the thyroid weights and the mean acinar cell heights increased proportionately until the tenth day. After the tenth day a widely disproportionate increase in the thyroid weights occurred (fig. 3).

2. The effect of thiouracil on the collection by the thyroid of radioactive iodine.—The chicks used in this experiment were selected from the same hatch as those used in the preceding one and the experiments were conducted at the same time. Ninety chicks were divided into three groups. The first group of twenty chicks served as a control group. The second group of twenty chicks received 1 unit of thyrotropic hormone daily for ten days and the third group of fifty chicks received 0.1 per cent thiouracil in their drinking water daily for ten days. On the tenth day radioactive iodine was given to ten animals from each of these three groups and at the same time medication was

withdrawn from the remaining chicks of each group. After intervals of twelve, twenty-four, forty-eight and 120 hours after the withdrawal of thiouracil groups of ten chicks were given radioactive iodine. The remaining control chicks (10) and those remaining in the group which had received thyrotropic hormone (10) until the tenth day were given radioactive iodine at the 120 hour interval. All animals were killed four hours after receiving the radioactive iodine.

The data comprising this experiment are summarized (table 2) and a graphic comparison of the changes in the mean collection of radio-

TABLE 2. COLLECTION OF RADIOACTIVE IODINE IN THYROIDS MADE GOITROUS BY THIOURACIL AND BY THYROTROPIC HORMONE

Hours after withdrawal of thiouracil	Animals	Collection of radioactive iodine, per cent
0	10	1.9±0.6[1]
12	9	9.0±2.5
24	11	38.5±3.0
48	10	52.4±3.8
120	10	32.5±2.1
Additional controls		
Hours after start of experiment		
0	10	29.6±4.2
120	7	15.9±2.3
Thyrotropic hormone		
Hours after withdrawal of thyrotropic hormone		
0	7	56.5±6.8
120	6	29.6±2.5

[1] The mean of each group is given with the standard error of the mean.

active iodine is included (fig. 4). The initial points on each curve show the percentage of injected iodine collected after ten days of treatment as indicated. In other words, thyroids which had been stimulated by injection of thyrotropic hormone collected 56.5 per cent of the injected dose, compared with 29.6 per cent collected by the controls and 1.9 per cent collected by the animals provided with thiouracil. After the withdrawal of thiouracil, however, the collection of radioactive iodine by the thyroids rapidly increased, being greater than that of the controls in twenty-four hours and even greater than the rate of collection shown by the glands stimulated with injected thyrotropic hormone in forty-eight hours. At an interval 120 hours after the withdrawal of medication from both groups, the percentages of recovered iodine had decreased proportionately. The thyroids of the control series at this point collected only 15.9 per cent of the io-

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dine injected, so that the stimulated glands were still capable of collecting more radioactive iodine than the glands of control animals.

COMMENT

The histologic appearance and course of development of anatomic changes following the administration of thiouracil (figs. 5, 6 and 7) have been compared with those following the injection of thyrotropic hormone. As previous studies have shown, pronounced changes of mean acinar cell height occurred within twenty-four hours after the injection of thyrotropic hormone. By contrast, no change either in the weight of the thyroid or in the mean acinar cell height took place for five days after the administration of thiouracil.

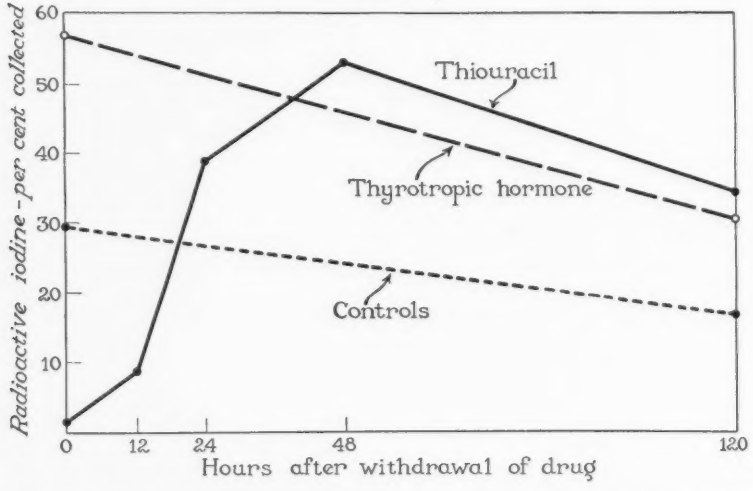

FIG. 4. The collection of radioactive iodine by thyroids stimulated for ten days with thiouracil compared with controls and with thyroids stimulated for ten days with 1 unit thyrotropic hormone. The initial points (at "0 hours") indicate the quantities collected at the time medication was withdrawn.

If one assumes that the flat-celled, colloid-filled acini of the thyroid of the chick must deplete themselves of stored hormone before thyroid insufficiency is pronounced enough to initiate compensatory hypertrophy after administration of thiouracil, then this lag is readily explained. It is striking that once the stimulation of the thyroids of chicks receiving thiouracil begins, the mean acinar cell height curve, as well as the increase of thyroid weight, closely duplicates the changes which follow the injection of thyrotropic hormone.

The disproportionate increase of the weight of the thyroid, as compared with mean acinar cell height, which occurs with continued stimulation with thyrotropic hormone, is just as striking with pro-

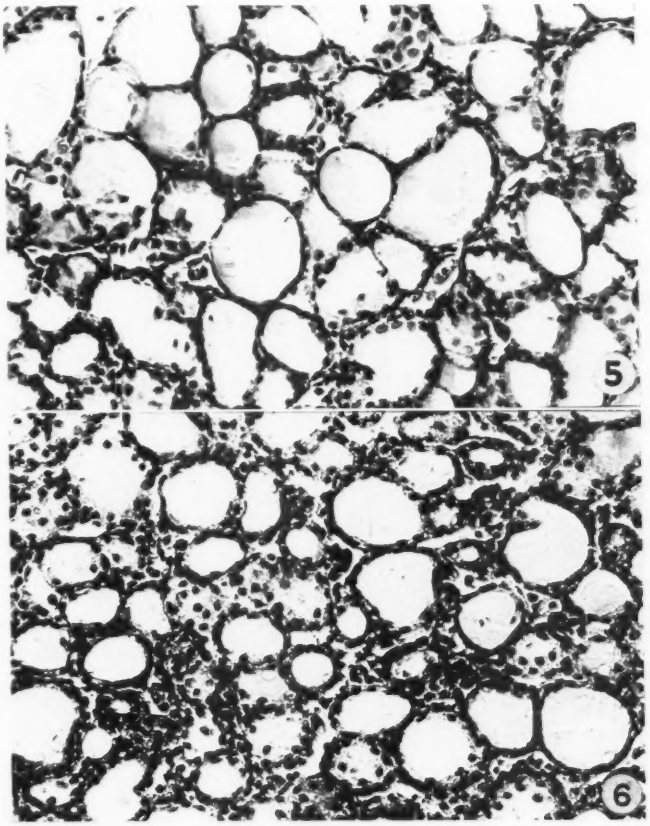

FIG. 5. Histologic appearance of control gland before stimulation with thiouracil was begun (hematoxylin and eosin ×300).

FIG. 6. Appearance of thyroid five days after administration of thiouracil was begun (hematoxylin and eosin ×300).

longed stimulation with thiouracil. In both instances it appears to represent the rapid development of true hyperplasia.

Thyroids made goitrous by continued administration of thiouracil were incapable of collecting more than a small fraction of the injected iodine when compared with control groups. Similar findings in the rat have been previously published (Rawson, Tannheimer and Peacock, 1944; Franklin, Lerner and Chaikoff, 1944). We have found that the withdrawal of thiouracil caused rapid release of this inhibition. Within forty-eight hours the thyroids which had been stimulated with thiouracil behaved essentially like those stimulated with thyrotropic hormone. So far as the capacity to collect radioactive iodine was con-

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cerned, both collected more of the injected dose than did controls. Since in each instance, the thyroids were larger than controls, this cannot be called a manifestation of hyperfunction. As shown elsewhere, if the capacity to collect radioactive iodine is expressed in terms of thyroid weight the collection of iodine by the stimulated glands is not increased.

In every respect in which they have been compared, the histologic and physiologic consequences of continued administration of thiouracil appear identical with the effects of injecting thyrotropic hormone In the presence of thiouracil the capacity to collect iodine is inhibited.

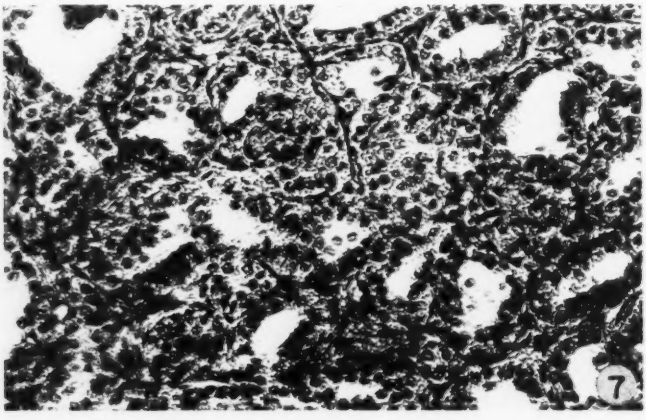

FIG. 7. Appearance of thyroid after ten days of treatment with thiouracil (hematoxylin and eosin ×300).

These findings are in full agreement with Astwood's hypothesis regarding the nature of the hyperplasia resulting from thiouracil and lend further support to the concept that its primary action may be to inhibit the collection of iodine by the thyroid.

SUMMARY

Following administration of thiouracil to the chick, no anatomic change was observed for five days, after which rapid development of hypertrophy and hyperplasia as measured by comparison of thyroid weight and mean acinar cell height took place.

Except for the lag in onset, the histologic changes following administration of thiouracil were found to be identical with those which follow the daily injection of thyrotropic hormone and developed in a similar manner.

The thyroid of the chick made goitrous with thiouracil showed a greatly diminished capacity to collect radioactive iodine. When thiouracil was withdrawn, such thyroids rapidly acquired a capacity to col-

lect radioactive iodine in much larger quantities than controls and in quantities similar to those collected by thyroids made hyperplastic by the injection of thyrotropic hormone.

The capacity of thiouracil to inhibit the collection of iodine by the thyroid may be its essential and primary effect. The development of thyroid hyperplasia which follows occurs later and appears histologically and functionally identical with that resulting from the injection of thyrotropic hormone.

We wish to acknowledge our gratitude and indebtedness to Dr. Robley D. Evans and his associates of the Radioactivity Center at the Massachusetts Institute of Technology for their active participation in these studies, to Dr. G. M. Higgins for many suggestions and the use of his laboratory at the Institute of Experimental Medicine of the Mayo Foundation and to Drs. J. H. Means, S. F. Haines and F. C. Mann for aid and encouragement.

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THE EFFECT OF THIOURACIL ON THE COLLECTION OF RADIOACTIVE IODINE BY THE THYROID OF THE CHICK[1,2]

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WE HAVE previously found (Larson, Keating, Peacock and Rawson, 1945) that the thyroid of the chick made goitrous by the administration of thiouracil in the drinking water had a diminished capacity to collect injected radioactive iodine. This effect was present only while thiouracil was being given; when the drug was withdrawn, the goitrous thyroids rapidly acquired a capacity to collect radioactive iodine in quantities even larger than controls.

Rawson, Tannheimer and Peacock (1944) have observed in the rat that goiters produced by the administration of thiouracil collected less radioactive iodine than did controls, whereas thyroid made goitrous by the administration of potassium thiocyanate collected a larger proportion of the injected dose than did controls. Franklin, Lerner and Chaikoff (1944) have made the same observation. In *in vitro* experiments by Franklin and Chaikoff (1943) and by Schachner, Franklin and Chaikoff (1944) it was found that sulfanilamide inhibited the formation of thyroxin and diiodotyrosine but did not limit the capacity of thyroid slices to collect inorganic iodine from the medium. Certain other substances did inhibit the collection of iodine by thyroid slices.

Astwood and Bissell (1944) have demonstrated that the iodine content of the thyroid of animals treated with thiouracil diminishes

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[2] Abridgement of part of thesis submitted by Dr. Larson to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of M.S. in Medicine.

rapidly as administration of the drug is continued. Baumann, Metzger and Marine (1944) have also found that the iodine content of thyroids made hyperplastic by administering thiouracil was reduced.

The evidence to date suggests that the primary effect of thiouracil is to prevent the collection of iodine by the thyroid. The hyperplasia and thyroid enlargement which takes place appear to be secondary results of stimulation with thyrotropic hormone. We have found that when thiouracil is administered to the chick there is a lag period of five days before evidence of thyroid stimulation appears. We therefore conducted the following series of acute experiments in order to observe the effects of thiouracil before sufficient time had elapsed to produce secondary histologic changes in the thyroid.

METHODS

White Leghorn cockerels were employed as experimental animals. Radioactive iodine having an atomic weight of 131 and a half life of eight days was used. The details of the technic employed have been described elsewhere (Keating, Rawson, Peacock and Evans, 1945).

RESULTS

1. The rate of collection of radioactive iodine by the thyroid of the chick.—Each of forty[3] chicks was given 1 unit of thyrotropic hormone[4] daily for four days. The chicks were given radioactive iodine and killed ten, thirty, sixty and 240 minutes later. The time intervals were accurately measured with a stop watch in each instance and each chick was dissected individually within thirty seconds of the time it was killed.

The data obtained in this experiment are summarized in table 1. When the data were plotted (fig. 1) it was apparent that maximal collection of radioactive iodine probably occurred about two hours after administration of the iodine. Twenty-five per cent of all the iodine that entered the thyroid did so within the first ten minutes. In all subsequent experiments animals were killed four hours after the injection of radioactive iodine, since this interval seemed likely to produce a maximal collection under the conditions imposed.

2. The effect of the injection of thiouracil on the collection of radioactive iodine by the normal thyroid of the chick.—Eighty chicks, four days old, were divided into two groups. Thirty chicks did not receive medication and served as controls, while the remaining fifty were given 10 mg. of thiouracil suspended in 1 cc. of distilled water and injected subcutaneously at the start of the experiment. Radioactive iodine

[3] The numbers given in the text are the numbers of chicks at the beginning of the experiments. The numbers given in the tables are the numbers of chicks that survived to the end of the experiments and on which the analyses are based.

[4] Thyrotropic hormone used was antuitrin T generously supplied by Dr. E. A. Sharp of Parke, Davis & Company. "Units" refer to Junkmann-Schoeller units.

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TABLE 1. RATE OF COLLECTION OF RADIOACTIVE IODINE

Minutes after injection of radioactive iodine	Animals	Collection of radioactive iodine, per cent
10	10	9.3 ± 1.4[1]
30	10	21.1 ± 3.5
60	10	32.0 ± 3.7
240	10	44.0 ± 4.2

[1] The mean of each group is given with the standard error of the mean.

was injected into the control animals in groups of ten at the start of the experiment and twenty-four and forty-eight hours later and into the treated animals also in groups of ten at one, six, twelve, twenty-

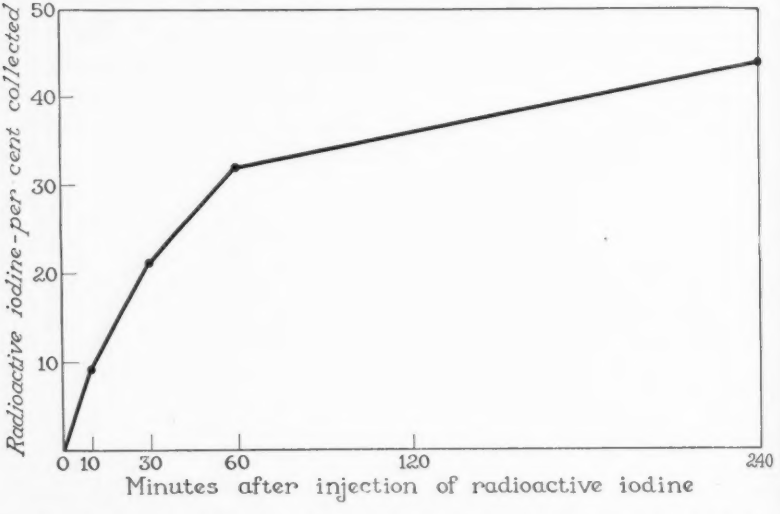

FIG. 1. The rate of collection of radioactive iodine by the thyroid of the chick. The chicks employed in this experiment were stimulated by the daily injection of 1 unit of thyrotropic hormone daily for four days.

four and forty-eight hours after the administration of thiouracil. All animals were killed four hours after receiving radioactive iodine.

The data are summarized in table 2 and are shown in figure 2. The control animals collected approximately 10 per cent of the injected dose throughout the experiment. One hour after the injection of thiouracil, the capacity of the thyroid to collect radioactive iodine had declined greatly; only 0.2 per cent of the injected dose was collected. The inhibition induced by the injected store of thiouracil gradually wore off and had disappeared at the end of forty-eight hours.

It will be noted that in this and subsequent figures the groups are plotted according to the time at which radioactive iodine was given (one, six, twelve, twenty-four and forty-eight hours) rather than the

TABLE 2. THE EFFECT OF THIOURACIL ON THE COLLECTION OF RADIOACTIVE IODINE BY THE THYROIDS OF NORMAL CHICKS

Hours after injection of thiouracil	Animals	Collection of radioactive iodine, per cent
0 (controls)	10	10.1 ± 1.8[1]
1	10	0.2 ± 0.1
6	10	0.5 ± 0.1
12	10	5.2 ± 1.5
24	10	5.1 ± 0.9
48	10	10.4 ± 3.1
Additional controls		
24	9	9.4 ± 3.2
48	10	11.3 ± 3.7

[1] The mean of each group is given with the standard error of the mean.

time at which the animals were killed (five, ten, sixteen, twenty-eight and fifty-two hours). The justification for this practice is found in the rate of collection of radioactive iodine shown by figure 1. Since 25 per cent of the injected radioactive iodine to reach the thyroid got there within the first ten minutes, it is apparent that in the present experiment the thiouracil was in fact effective at the time when radioactive iodine was injected, for if it were not, a larger proportion than 0.2 per cent of the injected dose would have been collected by the gland before the effect of the thiouracil began.

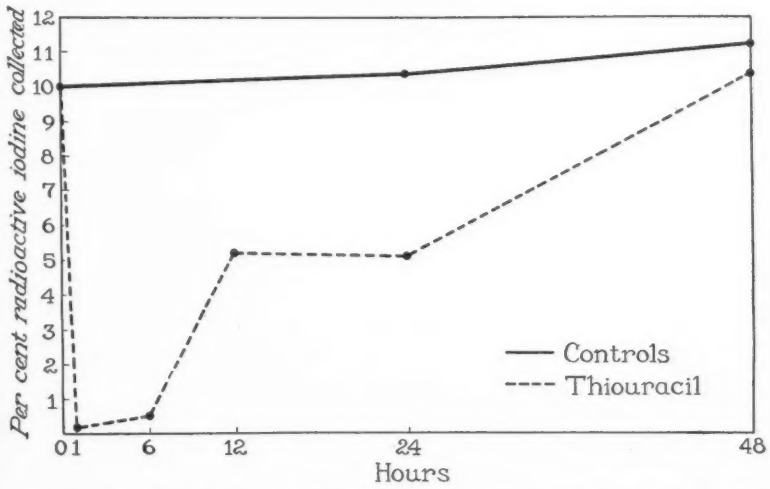

FIG. 2. The effect of thiouracil on the collection of radioactive iodine by the thyroids of normal chicks. Despite the fact that the thiouracil given (10 mg.) was in suspension it is apparent that its effect has materially worn off in twenty-four hours.

TABLE 3. THE EFFECT OF THIOURACIL ON THE COLLECTION OF RADIOACTIVE IODINE BY THYROIDS STIMULATED WITH THYROTROPIC HORMONE

Hours after injection of 10 mg. of thiouracil	Animals	Collection of radioactive iodine, per cent
0 (controls)	10	27.2±4.7[1]
1	10	0.7±0.1
6	9	1.2±0.2
12	10	5.0±3.1
24	7	23.4±3.0
Additional controls		
Hours after start of of experiment	Animals	Collection of radioactive iodine, per cent
24	10	29.6±3.8

[1] The mean of each group is given with the standard error of the mean.

3. The effect of thiouracil on the thyroid of the chick previously stimulated with thyrotropic hormone.—Sixty chicks received 1 unit of thyrotropic hormone subcutaneously daily for four days. Twenty-four hours after the last dose the experiment was begun. Twenty of these sixty chicks served as controls. Of these, ten were given radioactive iodine at the start of the experiment and ten were given the iodine twenty-four hours later. Forty of the sixty chicks were given 10 mg. of thiouracil suspended in 1 cc. of distilled water subcutaneously. Radioactive iodine was then injected at one, six, twelve and twenty-

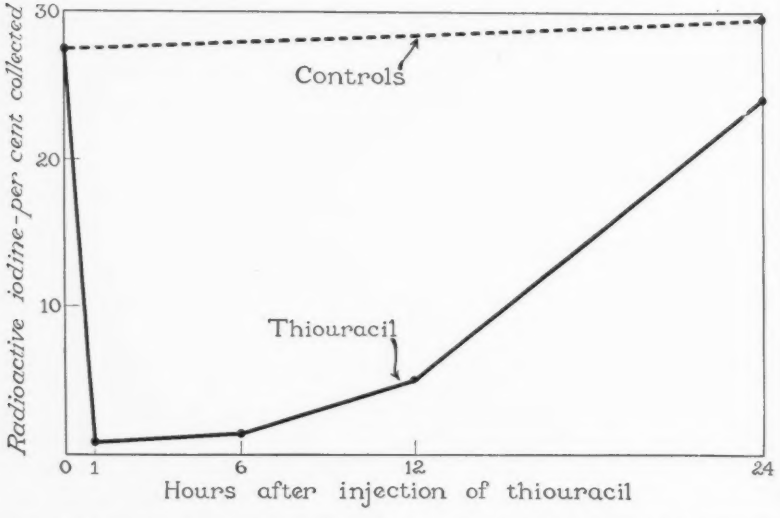

FIG. 3. The effect of thiouracil on the collection of radioactive iodine by the thyroid of chicks stimulated by previous injections of thyrotropic hormone.

four hours after thiouracil had been given. All control and test animals were killed four hours after receiving radioactive iodine.

The data summarized (table 3) and shown graphically (fig. 3). The thyroids stimulated by the injected thyrotropic hormone (controls) have an enhanced capacity to collect radioactive iodine when compared with the unstimulated thyroids in the preceding experiment (fig. 2). Despite this marked capacity to take up iodine, thiouracil produced within an hour an inhibition almost as great as that exerted on normal thyroids. The effect of the drug, however, had virtually disappeared after twenty-four hours despite the fact that the drug was injected as a suspension.

4. The effect of varying the dose of thiouracil on the collection of radioactive iodine.—Seventy chicks were given 1 unit of thyrotropic hormone daily for four days. The experiment was begun twenty-four hours after the fourth dose. The chicks were divided into seven groups of ten chicks each and given thiouracil in the following doses: 10.0, 5.0, 1.0, 0.5, 0.1, 0.01 and 0 mg. (controls). One hour after the administration of these graduated doses of thiouracil, radioactive iodine was injected and all animals were killed four hours later. All doses of thiouracil were given in 1 cc. of distilled water buffered to a pH of 7.3 and injected subcutaneously. All concentrations used were in solution excepting the largest one (10.0 mg.), which was in suspension.

The data are summarized (table 4) and shown graphically (fig. 4). In this graph the results are tabulated on a semilogarithmic scale, on which, except for an unexplained skew at the 1.0 mg. level, the resulting curve describes a fairly satisfactory straight line. The minimal dose of thiouracil which produced an effect appeared to lie between 0.01 and 0.1 mg. per chick; the maximal dose apparently lies between 1.0 and 5.0 mg. per chick.

5. The effect of thiouracil on the distribution of radioactive iodine in the thyroid.—Eight chicks were given 10 mg. of thiouracil. One hour later these chicks as well as eight controls were given radioactive iodine. All animals were killed four hours later. The thyroids were removed and those from four of the chicks which had received thiouracil were pooled in one digestion flask; the remaining four were pooled in a second one. The same procedure was followed with respect to the control group. The thyroids from four chicks which had not received radioactive iodine were placed in another digestion flask to which was added 1 cc. of the solution of radioactive iodine. This flask served as a blank.

The digestion mixture consisted of 10 cc. of 2N sodium hydroxide. All five samples were digested and separated according to the method described by Franklin and Chaikoff (1943). The quantity of radioactive iodine was determined (1) in an aliquot of the total digest; (2) in the fraction containing inorganic iodine and (3) in the residue containing organically bound iodine.

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TABLE 4. EFFECT OF VARYING DOSES OF THIOURACIL ON THE COLLECTION OF RADIOACTIVE IODINE

Dose of thiouracil, mg.	Animals	Collection of radioactive iodine, per cent
0 (controls)	10	44.0 ± 4.2[1]
0.01	9	47.4 ± 3.3
0.1	9	29.7 ± 4.9
0.5	9	21.2 ± 3.5
1.0	9	25.4 ± 5.1
5.0	9	1.4 ± 0.2
10.0	8	1.2 ± 0.1

[1] The mean of each group is given with the standard error of the mean.

The results are shown in table 5. In our hands this method of fractionation was not very satisfactory. From 7 to 30 per cent of the iodine present was lost in the process of separation and furthermore a significant portion of the inorganic iodine remained in the organic residue. Nevertheless the differences found were so striking despite these serious shortcomings that it was felt the results were significant and should be presented as supportive evidence. As can be seen from table 5, the inorganic iodine residue was of the same order of magnitude in the thiouracil series as in the control glands. The greater part of the iodine collected by the control glands was in the organic fraction, while the thyroids treated with thiouracil contained only a minute quantity of radioactive material in this fraction. If one considers

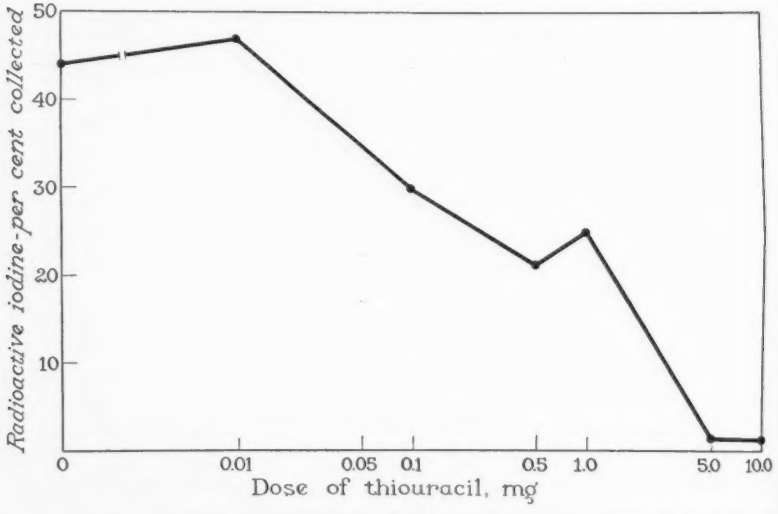

FIG. 4. The effect of varying doses of thiouracil on the collection of radioactive iodine. The graph is plotted on a semilogarithmic scale, on which, except for the skew at the 1.0 mg. level, the quantity collected appears to follow a straight line.

that a certain proportion of inorganic iodine may in truth, have remained behind in the organic residue as an artefact, as the fractionation of the blank would indicate, then it appears likely that the organic residue in the thiouracil series may actually be nil.

TABLE 5. EFFECT OF THIOURACIL ON THE DISTRIBUTION OF RADIOACTIVE IODINE IN THE THYROID

Specimen	Animals	Radioactive iodine per cent of injected dose		
		Total iodine	Organic iodine	Inorganic iodine
I. Thiouracil	4	1.2	0.1	1.0
II. Thiouracil	4	0.7	0.1	0.4
III. Control	4	6.7	4.7	0.6
IV. Control	4	9.7	6.9	0.8
V. Blank	4	100.0	9.2	84.7

It will be noted that the sum of organic and inorganic iodine does not equal the total iodine. The difference represents iodine lost in the process of fractionation. The iodine in the blank, which was subjected to the same chemical procedures, was obviously initially all in inorganic form since the radioactive iodine was added as sodium iodide to the digestion flask. Therefore the 9.2 per cent found in the "organic" fraction of the blank represents an error in the method under the conditions employed.

Further investigation of the fractionation of radioactive iodine collected by the thyroid is planned when a more accurate method of separation can be achieved.

COMMENT

Thiouracil has been shown in this study to possess the property of inhibiting the collection of radioactive iodine by the thyroid within an hour of the time of its administration. It is likely that this effect on the collection of radioactive iodine could be observed in much less than an hour but because of the technical difficulties involved no attempt was made to demonstrate the minimal time of action. Conversely, these experiments cannot be interpreted, except in a general way, as indicating the duration of action of thiouracil, since the dose of thiouracil employed was insoluble when injected and therefore, for a time at least, must have been present as a particulate depot under the skin. Despite this, it would appear that the effect of such a suspension does not greatly exceed twenty-four hours, so that the duration of action of thiouracil is probably brief.

In our previous study (Larson, Keating, Peacock and Rawson, 1945) we had shown that thiouracil reduces the capacity of the thyroid to collect radioactive iodine in the presence of hyperplasia induced by its continued administration. We have now shown that thiouracil can reduce with equal facility the collection of radioactive iodine by

the thyroid of the normal chick as well as by thyroids made goitrous by the injection of thyrotropic hormone. When submaximal doses of thiouracil are given, the degree of inhibition appears to vary as the logarithm of the dose. This suggests a means of comparing the goitrogenicity of other drugs having a similar mode of action.

It is interesting to compare the quantities of iodine still collected by the thyroid under conditions of maximal inhibition by thiouracil in the various experiments presented: (1) hyperplasia induced by thyrotropic hormone, 0.7 per cent and 1.2 per cent; (2) thyroid hyperplasia induced by thiouracil, 1.9 per cent and (3) normal thyroids, 0.2 per cent. These quantities are thirty to 100 times as great as the amount of iodine which one would expect to enter the thyroid on the basis of simple diffusion. If one assumes that these residues represent that fraction of the collected radioactive iodine which remained in the inorganic state, then the inference may be made that the diminished collection of iodine represents a complete inhibition of synthesis of organic iodine. The preliminary experiment on fractionation of iodine in the thyroid under the conditions employed supports this assumption.

These studies therefore confirm the conclusions of Astwood and his associates (1943) that thiouracil acts primarily by inhibiting the production of thyroid hormone and that the thyroid hyperplasia which follows is a secondary consequence of lack of thyroid hormone.

Harrington (1944) has recently presented a convincing summary of the probable course of events in the synthesis of thyroxine by the thyroid gland. He presented abundant evidence that diiodotyrosine is the natural precursor of thyroxin. The natural synthesis of thyroxin appears to involve two chief steps: first, the iodization of tyrosine and, second, the coupling of two molecules of diiodotyrosine to form thyroxin. The first of these requires the presence of free iodine and, since exogenous iodine has been shown to reach the thyroid as iodide, a strong oxidizing system is required to convert iodide to iodine before diiodotyrosine can be formed. Dempsey (1944) by new histologic technics has shown that a peroxidase is present in the thyroid cell and moreover has shown that it tends to disappear from the cell when thiourea is given. It thus appears possible that thiouracil and other substances having a similar mode of action may act primarily by inhibiting such a peroxidase system. An alternative hypothesis which would fit the facts is one which assumes that thiouracil acts somehow on iodides in such a manner as to prevent the thyroid from combining them in organic synthesis. In this regard, Astwood (1944) has recently observed that thiouracil can be shown *in vitro* to act as a reducing agent, converting iodine to iodide and preventing the oxidation of iodide to iodine.

SUMMARY

Anatomic changes in the thyroid of the chick following the administration of thiouracil did not appear until five days of treatment had elapsed. Inhibition of the capacity of the thyroid to collect injected radioactive iodine was shown to be at a maximum one hour after the initial injection of thiouracil.

Thiouracil inhibited the collection of radioactive iodine by the thyroid of the normal chick, by the thyroid made hyperplastic by the injection of thyrotropic hormone and by the thyroid made hyperplastic by the administration of thiouracil.

The effect of thiouracil on the collection of radioactive iodine was largely dissipated within twenty-four hours. The degree of inhibition produced by submaximal doses of thiouracil was a linear function of the logarithm of the dose.

The effect of thiouracil on reducing the quantity of radioactive iodine collected by the thyroid may be the result of suppression of synthesis of iodine-containing compounds in the thyroid.

We wish to express our gratitude and acknowledge our indebtedness to Dr. Robley D. Evans and his associates of the Radioactivity Center of the Massachusetts Institute of Technology for their active participation in these studies, to Dr. G. M. Higgins for many suggestions and for the use of his laboratory at the Institute of Experimental Medicine, Mayo Foundation, and to Drs. J. H. Means, S. F. Haines and F. C. Mann for aid and encouragement.

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THE EFFECT OF DIETARY PROTEIN ON ADRENAL WEIGHTS AND ON GROWTH AFTER UNILATERAL ADRENALECTOMY

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RECENT WORK on the adrenal cortex has emphasized its role in nitrogen metabolism particularly in regard to gluconeogenesis from protein. Long, Katzin and Fry (1940) found increased nitrogen excretion associated with the increased liver glycogen in fasted normal and adrenalectomized rats and mice when cortical extracts or adrenal steroids were given. Tepperman, Engel and Long (1943a) have suggested that hypertrophy of adrenal cortical tissue under a great variety of conditions may be due to an increased cortical activity associated with increased nitrogen metabolism.

The possibility that changes in the level of dietary protein might be associated with changes in adrenal weight has been explored by several authors. Tepperman, Engel and Long (1943b) report an adrenal weight increase in rats fed a high protein diet, whereas Ingle, Ginther and Nezamis (1943) report no significant change in adrenal weights in rats fed a diet with similar protein levels. This direct contradiction seemed to call for further study of the question and accordingly in the present work mice were fed high protein diets and studied with regard to body growth, adrenal weight, the volume of the cortex and medulla, and the histological appearance of the gland.

METHODS

Young male mice of the H strain bred in this laboratory and maintained on a Purina dog chow diet were used. The composition of the synthetic diets is shown in Table I, and the proportional distribution of calories in Table II. The analysis of the chow as reported by the manufacturer is crude protein >21 per cent, crude fat >4 per cent, crude fiber <6 per cent, and nitrogen free extract >46 per cent. All diets and water were given *ad libitum*. The chow and casein A diets are similar to those fed by Tepperman, *et al.*, (1943b) and the basal synthetic and the high protein I diets are modeled after those used by Ingle, *et al.* (1943).

The mice used were about 3 weeks old at the start of the experiment; precise ages differed for each group and are discussed under each experiment. At the conclusion of the feeding period the mice were weighed and killed by

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TABLE I. PERCENTAGE COMPOSITION OF SYNTHETIC DIETS

Constituent	Diet				
	BS	HPI	CA	MB	MHP
Casein[1]	20	65.8	78	10	77
Starch, powder, from wheat	20	—	—	40	—
Dextrin, powder, from wheat	10	—	—	27	—
Mazola Oil	17	11.9	5	—	—
Brewer's Yeast	8	7.9	10	8	8
Wheat Germ Oil	1	0.9	—	1	1
Cod Liver Oil	1	0.9	2	1	1
Salt Mixture[2]	5	3.1	5	3	3
Cellu Flour[3]	18	9.5	—	10	10

[1] Casein was "washed, from milk" (Fisher) and "technical" (Baker).
[2] Osborne and Mendel IV (1913).
[3] Chicago Dietetic Supply.
BS, basal synthetic.
HPI, high protein I.
CA, casein A.
MB, modified basal.
MHP, modified high protein.

TABLE II. PROPORTION OF TOTAL CALORIES DERIVED FROM PROTEIN, CARBOHYDRATE AND FAT IN DIETS DESCRIBED IN TABLE I

Caloric source	Diet				
	BS	HPI	CA	MB	MHP
Protein	24.1	66.8	79.3	15.6	89.3
Carbohydrate	31.6	2.6	3.3	76.3	3.0
Fat	44.3	30.5	17.5	8.1	7.7

For these calculations the casein was assumed to be pure protein, caloric value 4.4 cal. per gm., the yeast to contain 46.1 per cent protein, at 4.0 cal. per gm., 36.6 per cent carbohydrate, and 1.6 per cent fat. Carbohydrate and fats were taken at 4.1 and 9.3 cal. per gm., respectively. The cellu flour was assumed to contribute inert solids only.

bleeding, under ether or phenobarbital anesthesia, and the adrenals removed, cleaned of fat, and fixed immediately. Other organs were examined grossly and in some cases sections of the thyroid gland were made. No pathological changes were found.

Adrenals were fixed overnight in Bouin's fluid, washed in two changes of distilled water, drained on a clean filter paper and weighed separately or in pairs on a microbalance. Drainage times were kept nearly constant during weighing and weights were reproducible to 0.05 mgm. Paraffin serial sections were then made and stained with hematoxylin and eosin.

In the unilaterally adrenalectomized animals the left adrenals were removed under ether anesthesia through a lateral transverse incision about 1 cm. long over the lower margin of the spleen. The adrenal stalk was not ligated because in mice there is little tendency toward bleeding, and the incision was closed by two silk sutures through muscle and peritoneum and three or four in the skin. At autopsy adhesions were frequently found but they never involved more than the spleen and kidney.

The significance of the results was tested by applying the commonly used formulae for standard deviation and standard errors. The probability of oc-

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currence by chance was found using tables for the distribution of Student's t (Peters and Van Voorhis 1940). A probability of an occurrence by chance below 5 per cent is conventionally considered to indicate significant differences, and below 1 per cent, highly significant differences. All ± values given after data in the tables are standard deviations of the individuals.

Volume studies were made from serial 10μ sections of adrenals. Photomicrographs were made of every tenth section and projected from the negatives in an enlarger so that the image was 100 diameters actual size. Readings of the areas of the cortex and medulla were then taken with a planimeter, three readings for each area studied, and the total volumes computed.

EXPERIMENTS

Three series of experiments were performed with various modifications of procedure. The data are summarized in Table III. The three sets of experiments will now be described separately.

Series 1: Litters of mice with the mother were placed on the basal synthetic diet at 20–22 days of age to accustom them to the somewbat

TABLE III. EFFECT OF DIETS ON ADRENAL WEIGHTS AND BODY GOWTH IN INTACT AND UNILATERALLY ADRENALECTOMIZED MICE

Adrenals	No. of mice	Diet	Weeks fed	Adrenal wt.: body wt. ratio $\times 10^5$	P	Final body wt. (gms.)	P	Body wt. gain (gms.)	P
Series I									
IN	7	Chow	4	15.0±4.1	>5	23.2±2.4	>5	10.5±1.9	>5
IN	8	CA	4	16.7±2.6		22.9±1.1		11.6±0.7	
IN	6	BS	4	15.9±1.2	>5	24.0±1.5	>5	11.6±1.4	>5
IN	9	HPI	4	16.6±3.2		24.0±2.1		10.7±1.6	
Series II									
IN	7	BS	2	13.2±1.4	>5	22.9±3.9	>5	11.9±2.1	>5
IN	7	HPI	2	14.4±1.6		20.7±3.1		9.8±1.6	
LR	12	BS	2	08.0±0.9	>5	21.8±2.0	>5	9.5±1.4	1.2
LR	12	HPI	2	09.4±2.2		19.8±3.0		7.4±2.1	
Series III									
IN	15	MB	3*	16.0±2.0	>5	19.5±2.2	>5	10.3±1.5	>5
IN	16	MHP	3*	14.9±3.9		18.2±2.2		9.3±2.3	
LR	16	MB	3*	08.9±2.2	>5	20.0±2.4	1.3	10.4±1.8	.27
LR	16	MHP	3*	09.2±1.7		16.8±2.9		7.2±2.2	

IN, adrenals intact.
LR, left adrenal removed.
* Except 3 in each group, fed 2 weeks, and not included under body wt. or body wt. gain.
± Values are the standard deviation of individuals.
P, the per cent probability that the differences between the successive pairs of groups in the column to the left could have occurred as a result of chance distribution, using the formulae: Standard deviation of the individuals, $\sigma_1 = \frac{\sqrt{\Sigma x^2}}{N}$ and $\sigma_m = \frac{\sigma_1}{\sqrt{N-1}}$ while $t = \frac{m_1 - m_2}{\sqrt{\sigma^2_{m_1} + \sigma^2_{m_2}}}$. P was then obtained using student's tables for the distribution of t.

powdery food. On the third day the mice were weaned and on the following day the males were weighed and started on the experimental diets. Litter mates were distributed among the various diets when possible. These animals continued on the diets for 4 weeks. No significant differences were found in either the body weights, adrenal weights or in the histological appearance of the adrenals.

Series 2: Litters with the mother were placed on the basal synthetic diet at 20–22 days for 3 days. They were weaned on the third day and on the following day pairs of male litter mates matching each other in weight within 0.5 gm. were selected. Left adrenals were removed from some of these pairs. The experimental diets were continued for 2 weeks.

Mice fed the high protein I diet and with the left adrenal removed showed a significantly lower body weight gain, as shown in Table III, but the change was not marked enough to be significant in the final body weight mean. No significant change in the ratio of adrenal weight to body weight and no histological changes were observed.

Volume studies of the remaining adrenal glands were made on 7 pairs of the unilaterally adrenelectomized mice of this group. The data are given in Table IV. It can be seen that the ratio of cortex

TABLE IV. EFFECT OF HIGH PROTEIN DIET ON ADRENAL VOLUME IN UNILATERALLY ADRENALECTOMIZED ANIMALS

Diet	No. of mice	Total vol. (cu. mm.)	Cortex vol. (cu. mm.)	Cortex vol. (cu. mm.) / Body weight (gms.)
BS	7	$1.04 \pm .08$	$.880 \pm .060$	$.041 \pm .009$
HPI	7	$1.10 \pm .12$	$.956 \pm .122$	$.053 \pm .038$

volume to body weight showed no significant change, nor did the cortex volume or total adrenal volume change significantly. The correlation coefficient of adrenal weight to adrenal volume, an index of the reliability of these measurements, is $.727 \pm .069$. The correlation coefficient of adrenal weight to cortex volume is .736, a value not significantly different.[1]

Series 3: Modified diets were then used to give a greater difference in the per cent of protein, the protein replacing carbohydrate while

[1] The coefficient of correlation of adrenal weights with adrenal volumes was calculated from the formula of Peters and Van Voorhis (1940).

$$r = \frac{N\Sigma x'y' - \Sigma x'\Sigma y'}{\sqrt{[N\Sigma x'^2 - (\Sigma x')^2][N\Sigma y'^2 - (\Sigma y')^2]}}$$

Standard errors of the correlation were calculated from Van Uven's formula as given by Pearl (1940).

$$\sigma_r^2 = \frac{\dfrac{(\Sigma uv)^2}{\Sigma v - \Sigma u}}{(n-2)\Sigma u^2}$$

all other factors were constant. These diets contained only small amounts of fat, supplied in cod liver oil (1 per cent) and wheat germ oil (1 per cent). Both these substances contain a high percentage of unsaturated fats. Schneider, *et al.* (1939) report that wheat germ oil is adequate in doses of 4 mgm. per rat per day in curing acrodynia. Since our mice consumed well over 1 gm. of diet per mouse per day, we conclude there was no deficiency in anti-acrodynic activity.

TABLE V. HYPERTROPHY AFTER REMOVING LEFT ADRENAL

Change in adrenal weight					
Adrenals	No. of mice	Diet	Rt. adrenal wt. (mgm.)	Combined means	*P*
IN	7	BS	1.44	1.42 ± .21	<1
IN	7	HPI	1.39		
LR	12	BS	1.86	1.86 ± .28	
LR	12	HPI	1.86		

Change in adrenal weight per cent of body weight				
Adrenals	No. of mice	Diet	Rt. adrenal wt.-body wt. ratio $\times 10^3$	*P*
IN	7	BS	6.3 ± 0.4	2.5
LR	12	BS	8.0 ± 1.0	
IN	7	HPI	7.0 ± 1.0	<1
LR	12	HPI	9.4 ± 2.2	

IN, adrenals intact.
LR, left adrenal removed.
P, as in Table III.

Litters of mice with the mother were placed on the modified basal diet at 16–18 days and weaned 3 days later. On the day after weaning they were weighed and matched pairs were placed on high (MHP) and low (MB) protein diets. The left adrenal was removed in half of the pairs. With a few exceptions the mice remained on the diets 3 weeks. One unoperated mouse died for unknown reasons during the experiment.

No significant change was seen in the adrenal weight-body weight ratio, but body weights and body weight gains were reduced significantly in the operated animals fed the high protein (MHP) diet, as shown in Table III. No significant change was found in analyzing adrenal weights *per se* for this group, thus indicating that there was no actual change in adrenal weight which had been obscured by body weight variability.

Table V (animals from group 2) shows the significance of the compensatory increase in the weight of the right adrenal after the left gland had been removed. The average hypertrophy was 31 per cent

of the mean. A compensatory enlargement has been frequently observed, and an extensive bibliography is given by Tepperman, *et al.*, (1943a). Our observation of this hypertrophy shows that our technique was adequate to detect adrenal enlargement, and also that the adrenals of these mice were able to respond by enlargement to the appropriate physiological stimuli. Hence the failure of the glands to enlarge with the high protein diet was not due to intrinsic growth incapacity of the adrenal tissue.

DISCUSSION

Hypertrophy of the adrenal cortex due to a high protein diet was first reported by Fahr (1912). He described a 100 per cent enlargement in the adrenals when rabbits were fed a milk and egg diet, compared to controls of unspecified age, and sex, on an unspecified diet. The experimental animals suffered anorexia and lost weight, and exhibited lesions in the liver and vascular intima, suggesting that the conclusions were probably vitiated by extraneous factors. Whitehead (1932) observed an increase in the number of mitoses in the cortex when peptones were given in a single intraperitoneal injection, provided the mice survived more than a few hours. This has been cited by Sarason (1943) as evidence for hyperplasia due to protein metabolism, but might alternatively be considered as secondary to the toxicity of the injections. Harmful stimuli of various sorts have been reported to cause adrenal enlargement (Selye 1936).

Tepperman, *et al.* (1943b) described a 40 per cent increase in the weight of the adrenals in young rats (60 ± 2 gm. initial weight) fed diets of 60 to 78 per cent casein for 2 to 7 weeks, as compared with controls on chow. Ingle, *et al.* (1943) could not detect a difference in the size of the adrenals of rats (181 ± 1 gms. initial weight) fed a diet containing 67 per cent casein for one to four weeks, as compared with controls on various low casein synthetic diets.

Our mice were growing at a rate comparable to that of Tepperman's rats, and our highest protein diet (MHP) exceeded that used by Tepperman in its per cent of calories from protein, so that these factors do not account for our failure to observe a significant increase in adrenal size. Could the results of Tepperman *et al.* be due to some factor other than the total protein? In Tepperman's comparisons of chow or meat and casein A diets, there were a number of variables. There may have been differences in the content of minerals, vitamins, inert solids or specific amino acids. Although we did not observe a statistically significant difference in comparing chow with casein A, rats might be more sensitive than mice to one of the above factors.

Our results with mice completely confirm those of Ingle *et al.* (1943) with rats in regard to the absence of hypertrophy of adrenal tissue in animals on a high protein diet. Ingle *et al.* point out that their results are not entirely inconsistent with a small degree of hypertrophy, since a trend toward adrenal enlargement is seen, but is not

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statistically significant. Similarly our data would not reveal as significant an enlargement under ten per cent, but they are incompatible with a larger increase. However, preliminary data on female mice indicate that we cannot exclude the possibility of considerable adrenal hypertrophy in females on high protein diets.

Hypertrophy, however, may not necessarily mean an increased functional level of the cortex, but may be due to an increased survival time of cells which have already completed the secretory phase of their life cycle, as was emphasized by Bennett (1940). Although we did not detect adrenal hypertrophy in males on high protein diets, this does not imply that there may not have been increased functional activity, which one would expect from the work of Long, Katzin and Fry (1940). They found increased nitrogen excretion and hyperglycemia in rats and mice when cortical extracts were given. However, it is possible that the cortical hormones may be less concerned in the initial liver metabolism of dietary protein than in the utilization of tissue protein reserves during starvation or other stress (Ingle, 1942).

The data reveal a statistically significant decrease in the body growth over a period of two weeks in unilaterally adrenalectomized animals on diets containing 67 per cent of total calories as protein in comparison with 24 per cent. Furthermore, when the diets were modified to 89 and 15.6 per cent protein respectively the degree of growth retardation was increased after 3 weeks on the diets.

Ingle, Higgins and Kendall (1938) and Wells and Kendall (1940 a, b) reported that high levels of corticosterone inhibit growth in normal rats, and Marx, Simpson, Li, and Evans (1943) have shown the existence of an antagonism between pituitary growth hormone and the adrenal cortex hormone stimulated by adrenotropic hormone. However, these results certainly do not entirely explain the retarded growth observed in our mice, since a statistically significant retardation did not occur in the intact animals.

It would seem that the adrenals are normally able to handle adequately diets containing a very large proportion of protein. However, after removing one adrenal, the remaining adrenal apparently does not secrete sufficient hormone to provide both for normal growth and for the metabolism of quite a high dietary level of protein. Nevertheless, this situation does not apparently release an adequate growth stimulus for adrenal tissue. One may enquire whether the reason for this may not be that the adrenal secretion on the high protein diet contained relatively more of the sterols which favor gluconeogenesis and suppress body growth and relatively less of those sterols which maintain or favor growth. Marked differences in the growth effects of cortical sterols have been reported by Wells and Kendall (1940a), who found that desoxycorticosterone caused a gain in body weight slightly greater than normal, while corticosterone and C_{17} hydroxydehydrocorticosterone suppressed growth. If a change in relative amounts of secreted sterols occurs, in relation to the protein intake,

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it might not constitute an adequate stimulus for an increase in pituitary adrenotropic hormone, which would explain the failure to observe adrenal hypertrophy on the high protein diet.

In any event, since no growth retardation was observed in intact animals the result seems to be due to a partial adrenal insufficiency in some sense under the conditions of the extra stress attending the utilization of a high protein diet. Although it seems doubtful whether the secretory limit of the adrenal tissue would be reached without exciting the production of increased adrenotropic hormone and adrenal hypertrophy, it seems clear that the unilaterally adrenalectomized animals are at a disadvantage in utilizing a high protein diet. This might conceivably be due to the toxic effects of inadequately metabolized amino acids or their breakdown products, or to a restriction in the available calories due to a reduced rate of gluconeogenesis from the ingested protein. McCay, *et al.* (1939) have described a reversible retardation of growth in rats given a diet adequate in protein, vitamins and minerals but low in calories.

SUMMARY

No weight, volume or histological changes were found in the adrenals of young male mice fed high protein diets. After the left adrenal was removed, hypertrophy of the cortex of the remaining adrenal occurred, but this was of the same degree in animals fed high and low levels of protein.

Animals on an 89 per cent protein diet with the left adrenal removed showed a 31 per cent retardation in body growth, as compared with similarly operated controls on a 16 per cent protein diet. Unoperated animals showed no statistically significant growth retardation on high protein diets.

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NOTES AND COMMENTS

HYPOPHYSEAL STIMULATION BY INJECTION OF LIGHT-INACTIVATED ESTRONE[1,2]

As early as 1925 before any of the estrogenic substances had been isolated and characterized, Allen and Ellis (1925) found that ultraviolet light destroyed the estrogenic activity of relatively crude ovarian extracts. In this instance, the estrogenic extracts were irradiated in corn oil. Within the next year Jordan and Doisy (1926) found that a preparation of the estrogenic hormone from hog liquor folliculi was destroyed by exposure to diffuse daylight. In 1938, LaPorta and Lafratta apparently without knowledge of the earlier work, subjected purified estrone in alcoholic solutions to quartz mercury arc radiations for two hours. They described certain changes in the melting point, ultraviolet absorption spectrum, and other physical properties, but were unable to detect any inactivation of the estrone. These contradictory results were probably related to the differences in the methods used to irradiate, the solvents used, and the technic employed to bioassay the irradiation products. No definite attempt was made to verify either of these reports because they were not found until near the completion of the irradiation experiment mentioned in this paper. The claim of Allen and Ellis (1925) that estrogenic substances in crude ovarian extracts were inactivated by ultraviolet light was, however, substantiated by the following experiment. Crystalline estrone was dissolved in aqueous solution (1 cc. ethyl alcohol and 1 cc. 1/10 sodium hydroxide and 98 cc. distilled water) and irradiated for 8 to 10 hours with the unfiltered light from a quartz mercury arc. The estrogenic activity was thereby destroyed (Figge, 1943). The evidence for this was outlined in Table I.

Non-irradiated solutions of estrone (A) uniformly produced estrus in ovariectomized rats. The same solutions irradiated for 8 to 10 hours failed to induce estrus even in relatively large doses (6.4 μg.).

An attempt was made to test the relative carcinogenicity of the active and the irradiated inactive degradation product of estrone. To compare the carcinogenicity, 50 μg. doses of both compounds were injected into a large series of male and female mice. It had been planned to inject only frequently enough to keep the females that were injected with active estrone in constant estrus. Daily vaginal smears of some of these mice were therefore made. To

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[2] The estrone used was from a sample sent to Dr. Edgar Allen through the courtesy of Parke, Davis & Co.

ascertain whether this large 50 μg. dose of inactive estrone had influenced the estrous cycles of the non-ovariectomized mice in the experimental group, daily vaginal smears were also made of these mice. It was soon observed that the irradiated estrone (supposedly non-estrogenic) induced an estrous response that lasted 10 to 14 days or usually 2 to 3 days longer than in the controls which received a corresponding dose of non-irradiated estrone.

TABLE I. INFLUENCE OF UNFILTERED QUARTZ MERCURY ARC RADIATIONS ON THE ESTROGENIC ACTIVITY OF ESTRONE IN RATS

Solution	Estrone micrograms, dosage	Treatment	Number of rats injected	Number of rats positive[1]
1A	6.4	non-irradiated	138	138
2A	3.2	non-irradiated	32	32
3A	1.6	non-irradiated	20	20
4A	0.8	non-irradiated	28	22
5A	0.4	non-irradiated	20	9
6A	0.2	non-irradiated	11	1
		(8 to 10 hours)		
1B	6.4	irradiated	16	0
2B	3.2	irradiated	23	0
3B	1.6	irradiated	24	2[2]
4B	0.8	irradiated	16	1[2]
5B	0.4	irradiated	12	0
6B	0.2	irradiated	12	0

[1] Number of rats responding with cornified vaginal smears. All rats ovariectomized.
[2] The reaction in these exceptional cases not understood.

These same solutions of irradiated estrone were again tested on ovariectomized mice and found to be relatively inactive as compared to non-irradiated estrone.

Thus it was clear that the irradiated estrone had little or no effect in the absence of the ovary, but in the intact animal it was able to induce a strong estrous response. It was thought that perhaps the ovaries had the ability to reverse the changes effected by the action of light on the estrone molecule. The possibility of the conversion of the inactive estrogen back to active estrogen in the ovary was, however, not investigated and at present other possibilities appear to be more plausible.

With the technique which we used, neither the active nor the light-inactivated estrone had any detectible carcinogenic influence. The interest in the entire experiment flagged and was not revived until an article on the pituitary responses of mature male rats to an inactivation product of estrone appeared in the September issue of this Journal (Smith, 1944).

It was reported that Westerfeld's inactivated estrone stimulated the pituitary to produce and release larger quantities of gonadotropic and adrenotropic hormones than did estrone. This conclusion was reached mainly by studies on the comparative weight changes induced by the two substances in the adrenals and pituitaries in castrate male rats and in the decrease in transplantable gonadotropic activity of the pituitaries. The paradoxical estrous response in non-ovariectomized mice when injected with light inactivated estrone, as compared with the lack of estrous response when ovariectomized mice are injected with the same material, can readily be explained on the basis of a pituitary stimulation by the light-inactivated estrone. Such

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an intense stimulus, as this appears to be, probably induces the estrous response in the intact animals by releasing gonadotropic hormone to stimulate the ovary. The physiological significance and possible therapeutic applications of such inactivated estrogenic compounds are of interest. These data are presented as additional evidence in support of the concept stated by Smith (1944), that inactive degradation products of estrogens stimulate the production and release of gonadotropic factors from the anterior pituitary.

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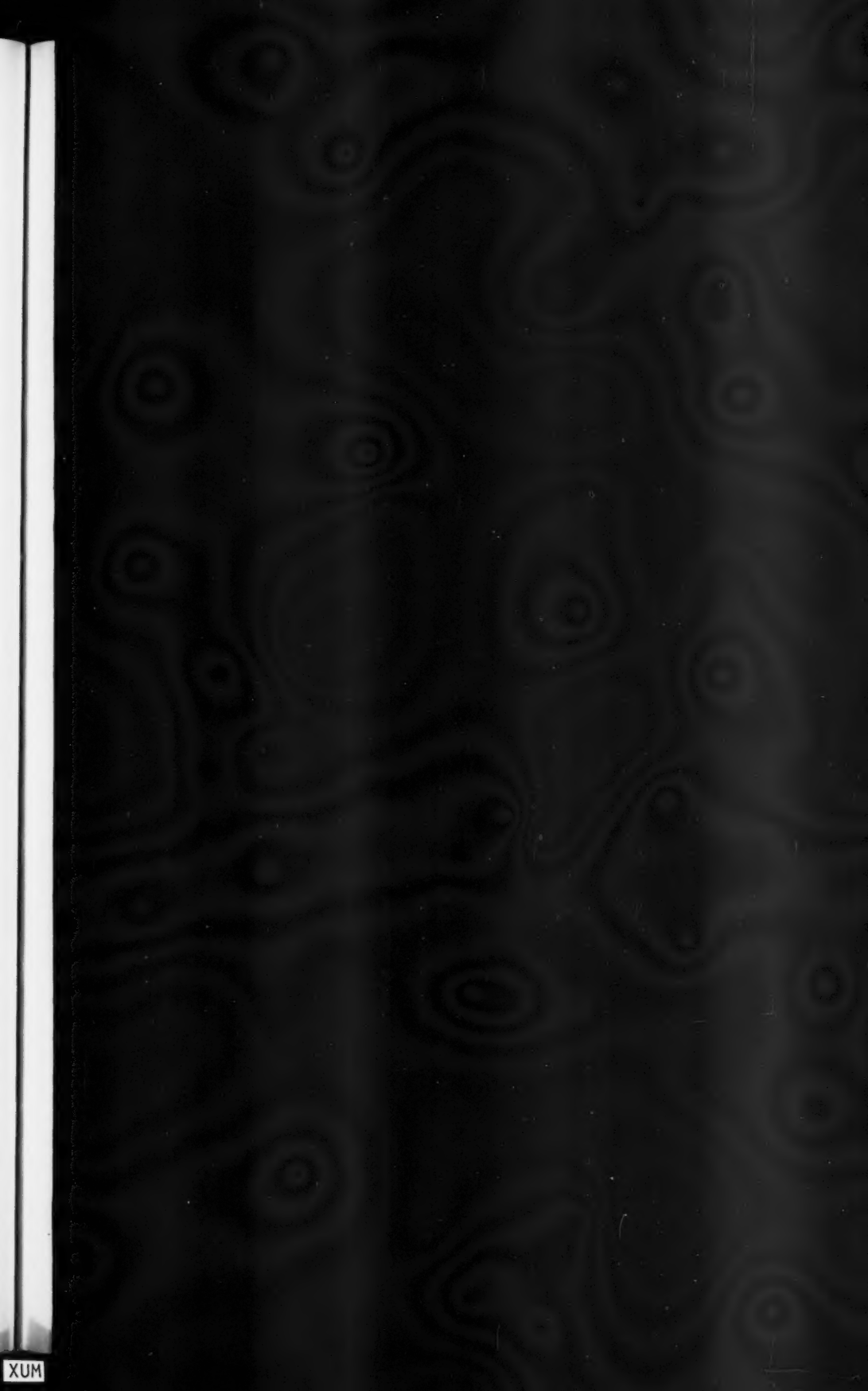
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